

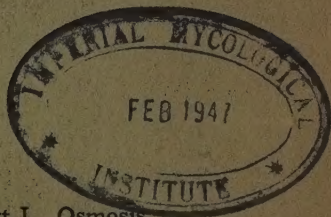
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The Movement of Materials Into Plants. Part I. Osmosis  
and the Movement of Water Into Plants . . T. C. BROYER 1

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## THE MOVEMENT OF MATERIALS INTO PLANTS PART I. OSMOSIS AND THE MOVEMENT OF WATER INTO PLANTS

T. C. BROYER  
*University of California*

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## INTRODUCTION

Numerous articles have been published on various phases of the movement of water into plants. The theoretical aspects of most such publications have been limited to explanations of specific experimental data. Only in text-books are comprehensive discourses presented, and frequently unity of thought is lost in the circuitous, interrelated discussions on several major physiological subjects. There is a need for a unified discussion of osmosis and its relation to water movement (water migration) into plants. The present theoretical treatment of the subject is presented in the hope of clarifying and extending present concepts in this important field of study. A new approach is attempted, citing examples, in accord qualitatively and quantitatively with experimental results, and based upon current definitions and principles of physics and physical chemistry (4, 7, 9, 11, 17-20, 35, 40, 43, 44, 61). A complementary treatise on the tendency for solute to move (solute migration) into plants will be given in Part II of this study.

## THEORETICAL ASPECTS OF OSMOSIS

The association of the various factors tending to cause water to move through the plant may be concisely developed from basic laws of physical chemistry, as follows:

*The Ideal Gas Law:* A perfect gas system is completely defined by the relationship (35, p. 63):

$$PV = nRT, \text{ where} \quad (1)$$

P is the pressure, due to the kinetic energy of the molecules, here expressed in atmospheres; V is the volume, in liters; n is the number of mols of gas; R is a "constant" factor, 0.08205 liter-atmosphere per degree; T is the absolute temperature.

*The Free Energy Concept:* The fundamental principle underlying the movement of materials is that each molecule possesses a total internal energy equal to the sum of its internal kinetic and potential energies (18, p. 57), and the molal (or partial molal) free energy is equal to the product of the mean free energy of the particles and the number of particles in one mole. A system is subject to spontaneous change if there is any conceivable process whereby the internal energy of the constituent molecules can be effectively reduced. The action, here especially that concerned with translation of the particle in space—its escaping tendency—which could be produced by such a conceivable process, is determined by the internal free energy of the individual molecules. The free energy of the particles may be modified by any change in condition of the external environment.

*Diffusion Defined:* Diffusion is the process whereby one substance moves into or through another in response to a difference of partial molal free energy<sup>1</sup>, tending toward equilibrium of escaping tendencies (35, pp. 179, 180).

*Osmosis Defined:* Osmosis is a process of diffusion of the component capable of free passage through a semipermeable membrane separating two components<sup>2</sup>, only one of which is capable of free passage through the boundary (54a). An osmotic system is a system in which the process of osmosis takes place. The osmotic relations of the system are determined by the constituent factors or influences involved in the consummation of the process of osmosis.

*A Semipermeable Membrane Defined:* A semipermeable membrane, in an ideal osmotic system, is a limiting layer, of infinitesimal thickness, separating two components, only one of which is capable of free passage through the boundary.

<sup>1</sup> As defined by Lewis and Randall (35), to include also the possible influences of variables other than pressure, temperature and composition. The phrase "free energy" will be used hereinafter with the understanding that it refers to the partial molal free energy of the molecules of a component of the system. Osmosis may be attended by modification of the  $p \times V$  product in either phase. The resultant action capacity between phases after any interval of time will in part depend on this product. Therefore, a free energy difference ( $\bar{f} - f^0$ ) is here expressed as that, corrected for any significant  $p \times V$  change in either phase, equivalent to a difference in "A" of Helmholtz (35, pp. 156-159).

<sup>2</sup> Since aqueous solutions are invariably involved in the two membrane-separated phases of biological systems, the terms "water" (solvent) and "solute" will be used, implying that the components are capable and incapable, respectively, of relatively free passage through the semipermeable membrane.

*Osmotic Specific Free Energies Defined:* Osmotic specific free energies ( $F$ ) are any action capacities of water associated with an osmotic system, expressed in positive dimensions of  $m L^{-1} t^{-2}$ , e.g., energy per unit volume, or pressure. An osmotic influx or efflux specific free energy is numerically equal to the difference in pressure, the osmotic pressure ( $P = p - p^\circ$ ), on a medium necessary to render the escaping tendency of water in a given state equal to that of water in a reference state. This pressure difference is related to the free energy of the water molecules in a dilute solution of solute and solvent contained in either phase of an osmometer, through the approximate relation:

$$\mp P = \mp (p - p^\circ) = \frac{\pm (\bar{f} - f^\circ)}{v^\circ (1.013 \times 10^9)} = F \quad (2)^3$$

where  $\bar{f}$  is the free energy of water in a given state other than the standard or reference state, here expressed in erg units;  $f^\circ$  is the molal free energy of water in its standard state, in ergs;  $v^\circ$  is the molal volume of water in its standard state, in liters;  $1.013 \times 10^9$  is the necessary conversion factor;  $p^\circ$  is the pressure on the water in its standard or reference state, in atmospheres; and  $p$  is the pressure on the medium in the given state, necessary to make  $\bar{f}$  equal to  $f^\circ$ , in atmospheres.

Since the molal volume of the component water in the standard state is a constant (here equal to 0.018 liter), it is evident from equation 2 that an osmotic influx or efflux specific free energy  $F$ , as well as an osmotic pressure  $P$ , is a measure of the quantity  $(\bar{f} - f^\circ)$  (see 35, p. 214; 18, pp. 100-104). In this treatise specific free energies are used as measures of the differences in free energy of the water molecules due to any constituent osmotic influence, in order to deal with dimensions commensurate with those generally employed by the biologist (compare 44). However, the action capacities may be

<sup>3</sup> Many physical chemists and biologists have related the escaping tendency of water through an interposed semipermeable membrane of an osmometer to the pressure difference  $p - p^\circ$ . However, since only one actual or exerted pressure is generally involved (i.e., hydrostatic) it is better, in order to avoid misconception, to express the osmotic values or action capacities, in terms of the differences in the free energy of the water molecules. This specific free energy  $F$  is expressed by the quotient  $\pm \frac{(\bar{f} - f^\circ)}{v^\circ}$ , related to, but not caused by, the pressure difference  $p - p^\circ$  as set forth in equation 2 (compare (54a)).

NOTE: A bar above the symbols  $\bar{f}$  and  $\bar{v}$  indicate partial molal quantities. The circular zero as super-script to the symbols  $f^\circ$  and  $v^\circ$  indicate the standard state.



expressed in fundamental dimensions of energy  $mL^2t^{-2}$ , (e.g., erg or calorie units). For the units used in this dissertation, the conversion is accomplished through the relation  $\pm (\bar{f} - f^\circ) = F (1.823 \times 10^7)$ , where  $F$  is expressed in atmospheres, and  $(\bar{f} - f^\circ)$  in ergs.

*Osmotic Pressure Defined:* Osmotic pressure is the pressure difference which must be applied to the two phases of an osmometer, under ideal conditions, to establish and/or maintain equilibrium of escaping tendency for water across the interposed semipermeable membrane (see 35, pp. 213-215). In equation 2 the osmotic pressure is represented by  $P$ , equal to the difference  $p - p^\circ$  (see Figure 1). (See the section entitled "On the Origin of Osmotic 'Solute'

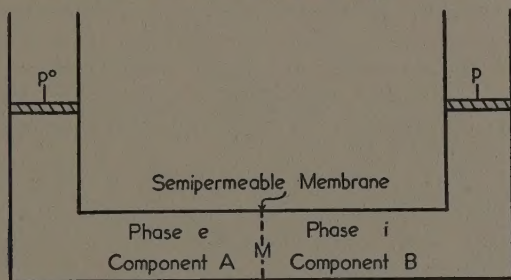


FIG. 1. Initial condition of an osmotic system in the ideal state.  
A = Component capable of free passage through the membrane.  
B = Component incapable of free passage through the membrane.  
M = Semipermeable membrane.

$$\pm \frac{(\bar{f} - f^\circ)}{v^\circ} = \mp p - p^\circ; \text{ for analysis, see text, page 4.}$$

Specific Free Energy", page 9, and compare with the solute specific free energy related to the pressure difference necessary to equilibrate the escaping tendencies for solute between two solution phases of an osmometer (Part II).)

*Osmotic Metabolic Specific Free Energy Defined:* In the plant, metabolic specific free energy,  $F_m$ , is any possible action capacity maintained directly through metabolism of the living organism which can be applied to tend to cause water to move, unilaterally, through the semipermeable membrane with or against the direction in which its concentration decreases<sup>4</sup>, by modification of the free energy of the water molecules.

<sup>4</sup> It has been suggested that osmosis should be termed "anomalous" where the flow of water through the semipermeable membrane is governed by

*Osmotic Non-metabolic Specific Free Energy Defined:* In the plant osmometer non-metabolic specific free energy,  $F_{nm}$ , is any possible action capacity (exclusive of hydrostatic specific free energy, which see) not directly maintained through metabolism of the living organism, which can be applied to tend to cause water to move through the semipermeable membrane with or against the direction in which its concentration decreases, by modification of the free energy of the water. Adsorption and imbibition are two typical processes or influences affording an action capacity of this type through lowering of the free energy of the water molecules at colloidal surfaces and within imbibants. Such processes imply the presence of matter other than molecules of solvent and solute in solution.

Osmotic "solute" specific free energy is one manifestation of a possible non-metabolic osmotic specific free energy, but because of its primary importance in the osmotic system it is discussed as a separate factor for water flow.

*Osmotic "Solute" Specific Free Energy Defined:* Osmotic "solute" specific free energy,  $F_s$ , is the action capacity tending to cause water to move through the semipermeable membrane either into a component incapable of free passage through the semipermeable membrane or into a solution of the two components (solvent water and solute). The water flows across the boundary into a solution within which the free energy of the molecules of the water has been lowered in relation to the concentration of the solute in the solution. In an ideal osmotic system the relationship between

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energies supplementing the osmotic "solute" specific free energy difference, as determined by the inequality of the solute concentration between the two phases of the osmometer. Anomalous osmosis is then designated "positive" where the flow is with the direction in which the concentration of water decreases; "negative" where the transport is against the direction in which the concentration of water decreases (9). In view of our limited knowledge of the factors involved, the term "anomalous" may justly be applied to this unknown osmotic mechanism. However, in order to maintain a consistent and more expressive terminology, the following definitions are proposed. "Metabolic specific free energy" tends to cause or maintain "osmotic, metabolic diffusion of water", the tendency toward flow being governed by energy supplementing the osmotic "solute" specific free energy difference, as determined by the inequality of the solute concentration between the two phases of the osmometer. The flow may be characterized as an "osmotic metabolic simple diffusion" where, and while, the flow is with the direction in which the concentration of water decreases; similarly, "osmotic metabolic accumulation" where the transport is against the direction in which the concentration of water decreases.



a measure of this specific free energy and the volume within either of the two phases involved, shows a formal resemblance to the ideal gas law (equation 1), *viz*:

$$F_s = \frac{n}{V} \cdot RT \quad (3)^5$$

where  $F_s$  is the specific free energy of the water, dimensionally and mathematically equal to the osmotic pressure, related to a difference in solute concentration in the two phases of the osmometer;  $V$  is the volume of solution; and  $n$  is the number of mols of solute in the solution (26).

Since  $\frac{n}{V} = C$ , where  $C$  is the volume molar (molarity) or, better, the weight molar (molality) concentration of solute in the solution,

$$F_s = CRT \quad (3b)$$

Assuming temperature changes are nil, the osmotic "solute" specific free energy may be measured as equal to the pressure which must be applied to produce a condition such that there is no tendency for the water to flow in either direction across the semipermeable boundary (see equation 2 and Figure 1). Employing the van't Hoff equation, 3 or 3a or 3b, as applicable in solution<sup>6</sup>, the osmotic "solute" specific free energy is a function of the concentration of solute in a solution. Thus  $RT$  is a coefficient for converting solute concentration into the related (antiphasically effective) osmotic "solute" specific free energy (see equation 3b).

*Hydrostatic Pressure Defined:* Hydrostatic pressure,  $P_h$ , is a pressure exerted uniformly in all directions from any point in a

<sup>5</sup> Some investigators (see 35, p. 236), on theoretical and experimental grounds, prefer the equation

$$F_s = \frac{N}{v^\circ} \cdot RT \quad (3a)$$

where  $N$  is the number of mols of solute in 1 mol of solvent, and  $v^\circ$  is the molal volume of the pure solvent.

<sup>6</sup> If the equation of van't Hoff is used to define the specific free energy and concentration relations of an ideal solution, then departure from the ideal behavior must be interpreted with respect to such factors as are likely to influence the osmotic "solute" specific free energy. He introduced a coefficient,  $k$ , into the equation, to correct for all types of deviation from ideal behavior without recourse to their origin. The osmotic "solute" specific free energy may be modified by the degree of dissociation of the molecules of the solute into constituent ions (57, p. 55) and by hydration (45, pp. 102, 103), in aqueous systems. Further, corrections may be applied for non-solvent volume and for forces of attraction between molecules in solution (10). For examples of possible deviation from the ideal, see data of Molz (47), Morse (48) and others (57, pp. 51-56).

fluid. Other factors being constant, any change in the hydrostatic pressure within a phase will directly modify the free energy of the component molecules, proportionately. If the pressure on, and free energy of, the molecules at any point are increased, they are increased everywhere throughout the fluid by the same amount. More specifically, within the osmometer the hydrostatic pressure,  $Ph_1$ , is the outwardly directed pressure of the inner component or solution on the membrane, which can be exhibited either by turgor in an inclosed phase or by a similar pressure in an open inner phase.

Such pressure, when positive, tends to cause water to move outward across the semipermeable boundary due to the increased free energy of its molecules in the inner phase of the osmometer (35, pp. 199, 243, 244). This pressure, positive or negative (18, p. 136), is equal to the algebraic result between that pressure ( $Ph_1'$ ) intrinsically associated with the medium in the inner phase of the reference system, caused by the restriction to expansion therein, and any extrinsic pressure ( $Ph_1''$ ) which may arise due to the application of energy of extra-phasic origin, *i.e.*, from outside the reference system. In an open inner phase, within the plant, the intrinsic hydrostatic pressure (with reference to that at the upper surface of the inner phase taken as zero, capillarity excluded) is caused by the weight of the column of fluid alone, the restriction to expansion being associated with the increasing total force (gravitation involved) in the liquid column. The extrinsic force may be any partial solution pressure (positive or negative, and usually the latter in the plant) which may arise within the osmometer due to the application of an action capacity to either the external or internal phase. In an inclosed inner phase, the intrinsic pressure, numerically equal to the membrane (wall) pressure, is caused by the restriction to expansion applied through extension of the elastic encircling membrane of the reference cell. This pressure may be positive, zero or negative. Here, as in the open inner phase, the extrinsic force may be any partial solution pressure, positive or negative, which may arise within the osmometer due to the application of an action capacity to either the external or internal phase. With a reference cell, a special extraphasic energy may be involved. Where it is surrounded by other cells, as in a tissue, an intercellular (wall) pressure, positive or negative, is effective.

*Hydrostatic Specific Free Energy Defined:* Hydrostatic specific

free energy,  $Fh_1$ , is that action capacity, caused by a hydrostatic pressure within an osmometer, which tends to cause water to move across the semipermeable boundary. Hydrostatic specific free energy is the resultant between the intrinsic hydrostatic specific free energy,  $Fh_1'$ , and any extrinsic hydrostatic specific free energy,  $Fh_1''$ . These constituent specific free energies are related to the comparable constituent hydrostatic pressures.

*Net Influx Specific Free Energy Defined:* The net influx specific free energy, NIF, is the difference in action capacity between the algebraic sum of the specific free energies tending to cause water to move into the system and those tending to cause water to move out of the system. The net influx specific free energy is equal to the sum of the influx specific free energies diminished by the sum of the efflux specific free energies, *i.e.*, net influx specific free energy = ( $\Sigma$  influx specific free energies) - ( $\Sigma$  efflux specific free energies) or  $NIF = \Sigma IF - \Sigma EF$ . (4)

*On the Origin of Osmotic "Solute" Specific Free Energy:* There are several views concerning the origin of osmotic phenomena expressed as pressures (20). Osmosis has been conceived generally as involving pressures of solute and/or solvent. This approach is illogical and unnecessary and leads to confusion over the mechanics of this process. Although the action capacities, tending to cause water to move through a semipermeable membrane of an ideal osmometer, may be expressed in dimensions of  $m L^{-1} t^{-2}$ , these specific free energy quantities are merely measures of the escaping tendency of solvent water molecules. Based on the free energy concept, rather than actual exerted pressures of the component molecules, the process is presented with more clarity. In the study of osmotic "solute" specific free energy, the essential feature is that the free energy of the solvent molecules is less in a solution than in the pure liquid at constant temperature and pressure; in other words, the transfer of water through an interposed semipermeable membrane from its pure (standard) state to that in a solution will result in a decrease of free energy. Such a flow will, therefore, always tend to occur whenever solvent and solution are brought together. Where they are separated by a semipermeable membrane, the water must flow into the solution until equilibrium is attained by the building up of a hydrostatic specific free energy, or its equivalent pressure, within the solution phase, ideally equal to the osmotic



"solute" specific free energy, and related to the concentration of solute in the solution, in accordance with the van't Hoff equation (see equations 3, 4). The existence of an osmotic "solute" specific free energy is the inevitable result of the introduction of a semipermeable membrane between a pure solvent and a solution, or between two solutions of dissimilar solute concentration, on account of the difference of the free energy of the solvent molecules in the two phases (compare the sections herein entitled "The Free Energy Concept", "Osmotic 'Solute' Specific Free Energy Defined", and "On the Origin of Osmotic 'Solute' Specific Free Energy" with the section entitled "The Free Energy of a Constituent Solute in a Solution", in Part II). See footnote 3.

*Deviations in the Plant, from the Ideal Osmotic System:*

I. Semipermeable membrane.

a. The thickness is finite.

1. Time is finite.

2. Semipermeability may be due to the restricted passage of the component (solute) not free to move through the limiting surface, through a capillary of, or through solution in, the bounding layer.

b. The membrane is never strictly semipermeable.

1. A metabolic specific free energy may be adapted to move unilaterally the component (solute) heretofore regarded as incapable of free passage through the semipermeable membrane through this differentially permeable boundary with or against (metabolic accumulation of solute) the direction in which its concentration decreases.

II. For each phase the change in volume is finite.

a. Time is finite.

b. After a finite time the system no longer involves pure components A (solvent, water) and B (solute), but rather component A (water) in phase e; and in phase i, a solution of A (water) and B (solute). (See Figure 1.) Further, since the membrane is not strictly semipermeable, the system may subsequently involve two solutions of the components, in phases e and i.

c. In the ideal aqueous osmotic system there should be no change in volume due to evaporation.

- III. Temperature changes are finite. However, in this treatise this factor is assumed to be non-variant.
- IV. Thermodynamic equilibrium, within the osmotic system as a whole, may be approached, but is probably never attained.
- V. The state of the system is not determined solely by the temperature, pressure and composition. Other independent variables, for example, gravitational and electrical fields, and surface effects, may be involved.

In the osmotic system of the plant, as indicated hereinbefore, the component capable of relatively free passage through the semi-permeable membrane is the solvent water. The component relatively incapable of free passage is the solute arising within the plant through metabolism or supplied to the organism from without. In the imperfect osmotic system of the plant, we have to deal not with two pure components, but with aqueous solutions, or colloidal systems, in the two membrane-separated phases.

*Comparison Between Two Alternative Aspects of the Fundamental Equation for Water Movement in an Osmometer:* There are two alternative methods of viewing the osmotic relations of plants, both based upon the fundamental physico-chemical principles of osmosis. The first method is useful only in a purely mathematical analysis of the system. This scheme has been developed through the cumulative efforts of a number of investigators, most recently advanced by Meyer and others (43-45). The second, alternative method, is useful in a purely mathematical analysis of the system and also serves to present, in a simple manner, the osmotic relations in graphic form, extending the diagrams of Thoday and Höfler (59, 27).

Consider the process in which water moves in an osmometer from a point external to the interposed membrane, to a point internally, *i.e.*,  $\text{H}_2\text{O}$  (external)  $\xrightarrow{\text{influx}}$   $\text{H}_2\text{O}$  (internal) (see 18, pp. 100-104). The measure of the tendency for water to move is given by the difference between the free energy of water in the internal phase and that in the external phase of the osmometer,  $(\bar{f}_i - \bar{f}_e)$ . If this difference is negative in sign, water will tend to move inward, as written. Thus, if it is desired to know whether or not water will tend to move across the membrane, the quantity  $(\bar{f}_i - \bar{f}_e)$  must be determined.

In practice the free energies themselves are not determined, but the difference between the free energy in a given state and the free energy in a reference or standard state. For water the standard state is customarily chosen as that of pure water under standard conditions. If by thermodynamic methods the quantities  $(\bar{f}_1 - f^\circ)$  and  $(\bar{f}_0 - f^\circ)$  are determined, then the difference between these quantities is  $(\bar{f}_1 - \bar{f}_0)$ .

As will become clear there is an entirely equivalent method of measuring the tendency for the above process to occur. This method involves the measurement of the osmotic specific free energies ( $F$ ) in each phase, distinguishing between those constituent influences which tend to decrease the free energy of the component water in the given state with respect to that in the reference state  $(-\Delta f)$ , and those which correspondingly tend to increase the free energy  $(\Delta f)$ . The presence of solute or a hydrophilic imbibant in an aqueous medium will tend to lower the free energy of the solvent water. A hydrostatic pressure, or turgor in an enclosed phase, will raise the free energy above that in the reference state. This physico-chemical analysis of the osmotic specific free energies involved in the system will be presented by means of equations which apply only for dilute solutions and low pressures.

The osmotic "solute" specific free energy is defined as equal, both dimensionally and mathematically, to the increase in pressure on a solution necessary to make the free energy of the solvent in the given state, the same as that of the pure solvent. For dilute aqueous solutions, the osmotic "solute" specific free energy ( $F_s$ ) is given by the equation:

$$F_s = p - p^\circ = \frac{-(\bar{f} - f^\circ)}{v^\circ} \quad (5)$$

where  $\bar{f}$  is the free energy of water in the solution;  $f^\circ$  is the free energy of water in the pure state;  $v^\circ$  is the molal volume of pure water;  $p^\circ$  is the pressure on the water in the pure state; and  $p$  is the pressure necessary to make  $\bar{f}$  equal to  $f^\circ$ .

Since the molal volume of pure water is a constant, it is evident from equation 5 that the osmotic "solute" specific free energy ( $F_s$ ) is a measure of the quantity  $(\bar{f} - f^\circ)$ . In the absence of other osmotic influences on the system, it follows that

$$F_{s1} - F_{s0} = \frac{-(\bar{f}_1 - \bar{f}_0)}{v^\circ} \quad (6)$$



where  $F_{s_i}$  and  $F_{s_e}$  are the antiphasic osmotic specific free energies related to the presence of internal solute and external solute, respectively. As previously stated,  $(\bar{f}_i - \bar{f}_e)$  is a measure of the tendency for water to move across the membrane. From equation 6 it is apparent that the quantity  $(F_{s_i} - F_{s_e})$  is also a measure of the tendency for water to move. A positive value for  $(F_{s_i} - F_{s_e})$  indicates a tendency for water to move inward, *i.e.*, an influx of water.

The above method may be applied to cases where either or both of the phases of the osmometer may be subjected to other osmotic influences, isothermally. The relationships apply only for dilute solutions and low pressures. An increase in pressure on a phase of the system ordinarily is accompanied by an increase in the free energy of water. For water the change in free energy due to an imposed pressure is proportional to the change in pressure, *i.e.*,

$$F_{(\Delta f)} = -(p - p^\circ) = \frac{(\bar{f} - f^\circ)}{v^\circ}. \quad \text{On the other hand, the change in free}$$

energy of water due to the presence of dissolved material is proportional to the negative of the osmotic "solute" specific free energy,

$$\text{i.e., } F_{(-\Delta f)} = (p - p^\circ) = \frac{-(\bar{f} - f^\circ)}{v^\circ}. \quad \text{The two equations, therefore,}$$

have the same form, but opposite signs. If the specific free energies, equal to the imposed pressures necessary to make  $\bar{f}$  equal to  $f^\circ$ , are designated by the symbol  $F_{(-\Delta f)}$  for constituent influences lowering the free energy of the solvent water, and by the symbol  $F_{(\Delta f)}$  for constituent influences raising the free energy, it follows that

$$(\Sigma F_{(-\Delta f)} - \Sigma F_{(\Delta f)}) = \frac{-(\bar{f} - f^\circ)}{v^\circ} \quad (7, \text{ see equation 2})$$

and for the two phases, that

$$\begin{aligned} & ((\Sigma F_{(-\Delta f)}) - (\Sigma F_{(\Delta f)}))_i - ((\Sigma F_{(-\Delta f)}) - (\Sigma F_{(\Delta f)}))_e = \frac{-(\bar{f}_i - \bar{f}_e)}{v^\circ} \\ & = \text{DPDD} = \text{NIF} \end{aligned} \quad (8)$$

Thus the expression,  $((\Sigma F_{(-\Delta f)}) - (\Sigma F_{(\Delta f)}))_i - ((\Sigma F_{(-\Delta f)}) - (\Sigma F_{(\Delta f)}))_e$ , is also a measure of the tendency for water to move. A positive value for this quantitative difference indicates a tendency for water to move inward, *i.e.*, an influx of water. Two typical examples of the measurement of the tendency for water to move may be taken from equations 12 and 36, to be discussed later. For

example, the tendency for water to move inward =  $((F_{s_i}) - (F_{h_i})) - (F_{s_e})$ . (9a)

Again, the tendency for water to move inward =  $((F_{s_i}) - (F_{h_i})) - ((F_{s_e} + F_{nm}) - (F_m))$ . (9b)<sup>7</sup>

In the terminology of Meyer these tendencies for water to move inward are expressed in each case by the difference between the algebraic sum of the diffusion pressure deficits for water associated with internal factors, and the algebraic sum of the diffusion pressure deficits for water associated with external factors, where the difference is represented by the equations:

$$DPDD = (\sum DPD)_i - (\sum DPD)_e \text{ and} \quad (10)$$

$$DPDD = (OP + (-TP))_i - (OP)_e; \text{ or} \quad (10a\text{---see equations 9a and 12})$$

$$= (OP + \dots + (-TP))_i - (OP + \dots + \dots)_e. \quad (10b\text{---see equations 9b and 36})$$

In order to express these specific free energies in equations and in graphic form, where the tendencies for water flow may be followed in relation to the changes in the external and internal specific free energies and the changes in the internal volume (the latter heretofore considered constant), an alternative scheme was developed. Here, the difference between the algebraic sum of the action capacities tending to cause water to move inward and the algebraic sum of those tending to cause water to move outward is a measure of the net tendency for water to move inward across the membrane. The osmotic specific free energies, regrouped in the equation of net tendency for water flow, are expressed in terms of the changes in the actual osmotic specific free energy of the water molecules, due to existing action capacities related to the constituent influences in an osmometer phase. In other words, the constituent

<sup>7</sup> It may be observed that the metabolic specific free energy factor tending to cause the component (water here) to move inward in the system is designated as an influence tending to increase the free energy of the component in favor of the external phase. The over-all movement of the component against the direction in which its concentration decreases, is in the direction opposite to that in which a flow "normally" would occur and is therefore associated with an apparent increase in free energy between the two phases. The law of tendency toward flow with the direction in which the specific free energy of the component decreases is maintained by asserting a complementary decrease of free energy within the cytoplasm, more than sufficient to compensate for the increase in question (35, pp. 120, 121). Oxidative catabolism would be required in this way for metabolic accumulation of water.

specific free energies of the system, related as expressed in equation 8, are regrouped to accord with the net influx specific free energy equation 4 (see p. 9). Thus, the equation

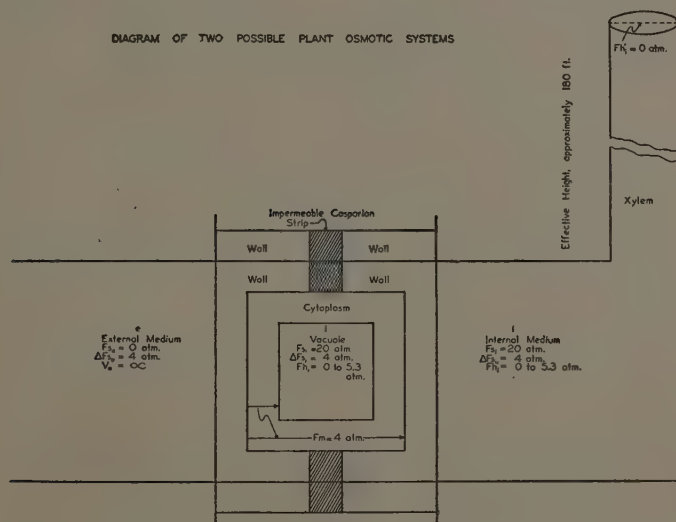
$$\text{NIF} = \Sigma \text{IF} - \Sigma \text{EF} \quad (4)$$

is equivalent to

$$\text{NIF} = ((\Sigma F_{(-\Delta f)})_i + (\Sigma F_{(\Delta f)})_e) - ((\Sigma F_{(-\Delta f)})_e + (\Sigma F_{(\Delta f)})_i). \quad (4a)$$

#### MOVEMENT OF WATER IN THE PLANT: THE ALTERNATIVE GRAPHIC SCHEME

In the water relations of the plant<sup>8</sup> (34, 37), two reference



In the initial state the volume of the external medium is assumed to be infinite, that of the internal medium, relatively small. Hydrostatic specific free energies assumed to be zero, initially.

FIG. 2. Diagram of two possible plant osmotic systems. The two systems represented are, first, the simplified, integrated osmotic system, external medium (water, nutrient solution, or soil medium)—membrane (endodermal cytoplasm or protoplasm of a comparably structured cell series)—internal medium (xylem solution, *etc.*); and second, a reference cell, external medium (nutrient solution or intercellular solution)—membrane (cytoplasm)—internal medium (vacuolar solution).

<sup>8</sup> Water movement by mass flow along continuous water columns (uninterrupted by differentially permeable septa) from root to shoot, similarly governed by the free energy difference of the solvent molecules, involving evaporation and cohesion, will not be discussed in detail (see 15, 28, 34, 45, 60; compare 51, 53).



TABLE I

SPECIFIC FREE ENERGIES INVOLVED IN THE MOVEMENT OF WATER INTO  
PLANTS UNDER NATURAL ENVIRONMENTAL CONDITIONS\*  
Net Influx Specific Free Energy =  $\Sigma$  Influx Specific Free Energies -  
 $\Sigma$  Efflux Specific Free Energies NIF =  $\Sigma$ IF -  $\Sigma$ EF

Water Influx Specific Free Energies (IF)	Water Efflux Specific Free Energies (EF)
<p>A. Osmotic "solute" specific free energy (antiphasically effective), related to the presence of internal solute†, arising through solute (electrolyte and/or non-electrolyte) influx (by simple diffusion, Donnan diffusion, exchange adsorption or metabolic accumulation) into the medium bathing the inner surface of the cell (endoplasm), or of the endodermis or comparably structured cell series. <math>F_{s1} = IF</math>.</p> <p>B. "Negative" hydrostatic specific free energy resulting from hydrostatic tension (negative pressure), internal, <math>-F_{h1} = \Delta IF</math>. Intrinsic hydrostatic specific free energy plus extrinsic hydrostatic specific free energy, i.e., <math>F_{h1} = F_{h1}' + F_{h1}''</math>.</p> <ol style="list-style-type: none"> <li>1. Open inner phase: here <math>F_{h1}'</math> is positive and <math>F_{h1}''</math> is negative; that is, a partial solution tension exists exceeding <math>F_{h1}'</math> numerically.</li> <li>2. Inclosed inner phase: here <math>F_{h1}'</math> is zero or negative (resulting from intracellular tension, membrane ("wall") tension). <math>F_{h1}''</math> is zero or negative, usually varying directly as <math>F_{h1}'</math> (resulting from intercellular tension).</li> </ol> <p>C. Hydrostatic specific free energy resulting from hydrostatic pressure, external. <math>F_{h0} = \Delta IF</math> (<math>F_{h0} = F_{h0}' + F_{h0}''</math>).</p> <p>D. Metabolic specific free energy, inwardly directed; arising within the living cytoplasm, <math>F_m = \Delta IF</math>.</p> <p>E. Non-metabolic specific free energy, inwardly directed. <math>F_{nm} = \Delta IF</math>.</p> <ol style="list-style-type: none"> <li>1. Internal imbibant (<math>F_{nm1}</math>), antiphasically effective.</li> </ol>	<p>A. Hydrostatic specific free energy resulting from hydrostatic pressure, internal. <math>F_{h1} = EF</math>. Intrinsic hydrostatic specific free energy plus extrinsic hydrostatic specific free energy; i.e., <math>F_{h1} = F_{h1}' + F_{h1}''</math>.</p> <ol style="list-style-type: none"> <li>1. Open inner phase: Here <math>F_{h1}'</math> is positive and <math>F_{h1}''</math> is either zero or negative; that is, a partial solution tension less numerically than <math>F_{h1}'</math> may or may not exist.</li> <li>2. Inclosed inner phase (resulting from hydrostatic pressure): Here <math>F_{h1}'</math> is positive or zero (resulting from intracellular pressure, membrane ("wall") pressure). <math>F_{h1}''</math> is positive or zero, usually varying directly as <math>F_{h1}'</math> (resulting from intercellular pressure).</li> </ol> <p>B. Osmotic solute specific free energy (antiphasically effective) related to the presence of external solute. <math>F_{s0} = \Delta EF</math>.</p> <p>C. Metabolic specific free energy, outwardly directed. <math>F_m = \Delta EF</math>.</p> <p>D. Non-metabolic specific free energy, outwardly directed. <math>F_{nm} = \Delta EF</math>.</p> <ol style="list-style-type: none"> <li>1. External imbibant (<math>F_{nm0}</math>), antiphasically effective.</li> </ol>

\* Unless otherwise specified (see sections on effect of suction and miscellaneous effects), all pressures are above the reference atmospheric pressure,

systems will be examined (see Figure 2): first, the simplified, integrated osmotic system (6, 12, 52), external solution—membrane (endodermal cytoplasm or protoplasm of a comparably structured cell series)—internal solution; and second, a reference cell, external solution—membrane (cytoplasm)—internal solution (vacuole). Although probably not strictly comparable, these will be discussed as similar systems. Modifications of similarity will be presented. The dynamics of osmotic water movement governed in any case by the over-all free energy difference of the water molecules in the system are expressed, in a summarized form, by the relations in Table I. The net free energy of the water molecules tending to cause its movement into the plant osmometer, equals the free energy of the water molecules in the external phase diminished by the free energy of the water molecules in the internal phase<sup>9</sup>. This treatise presents the constituent osmotic specific free energies which are measures of the free energies of the water molecules in the two phases, regrouped into their categories as influx or efflux specific free energies.

*The Fundamental Equation* (see 64, p. 437): From equation 3b it may be seen that the greater the concentration of solute in a solution, the greater is the antiphasic osmotic "solute" specific free energy. Since the free energy of the water molecules is lowered by this means in the internal phase of an osmometer, the efflux specific free energy for water is reduced and the influx specific free energy for water is proportionately increased. Water will enter the osmometer due to the higher solute concentration of the internal

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which is real and equal throughout the systems described. Here, the use of a reference pressure and the term net-influx specific free energy are not open to the criticism posed by Crafts (11, p. 387) in connection with other modes of presentation of osmotic relations.

† The externally effective osmotic "solute" specific free energy is related to the antiphasic solute concentration, in the internal phase (see equations 3 and 3a). The internally effective osmotic "solute" specific free energy is similarly related to the solute concentration in the external phase of the osmometer, bathing the ectoplasm of the reference cell or outer surface of the endodermis or cell series.

<sup>9</sup> The external or internal phases of either of the reference osmometers may be considered as individual units (see footnote 10) with respect to the free energy of the water. At equilibrium, the free energy of the water is constant throughout a phase, with relation to a reference level in space, regardless of its shape or size. At a lower position in space within the phase, the free energy is diminished on account of the influence of gravity, but it is increased by the intrinsic hydrostatic pressure (due to the mass of the column alone); these two influences just offset one another (35, pp. 242, 243).

phase<sup>10</sup>. If in an open system a pressure head of fluid develops, due to the entry of water, the influx specific free energy, related to the solute concentration difference alone, will be diminished by a counter specific free energy within the osmometer. This latter specific free energy results from the hydrostatic pressure due to the weight of the heightened column of the inner solution. This complementary specific free energy within the osmometer is termed the intrinsic hydrostatic specific free energy ( $Fh_1'$ ). Water will enter until this specific free energy in the internal solution counterbalances the osmotic specific free energy in the external phase ( $Fs_1$ ), related to the presence of internal solute. In an enclosed osmotic system, exemplified by an isolated reference cell, a counter pressure on the membrane is also produced. Instead of a hydrostatic pressure due to the weight of a column of liquid, an outwardly directed hydrostatic pressure is involved, counteracted by the restricted expansion imposed by the elastic membrane encircling the inner phase. As in the open inner phase system, water will enter the inclosed phase until the internal hydrostatic specific free energy, resulting from the internal hydrostatic pressure on the membrane, counterbalances the osmotic specific free energy related to the presence of internal solute.

On osmotic entry of water, to satisfy an existing free energy difference, the net influx specific free energy is diminished. The osmotic specific free energy related to internal solute is decreased by dilution of the internal medium, and the hydrostatic specific free energy is concurrently increased. The relative changes in the latter quantities will depend upon the extensibility of the inner phase. The lower the extensibility of the inner phase, the smaller will be the water influx accompanied by a relatively smaller decrease in the osmotic "solute" specific free energy and a relatively larger increase in the intrinsic hydrostatic specific free energy.

Ideally, the relations between changes in the relative volume of the inner phase of an osmometer and the hydrostatic specific free

<sup>10</sup> When the solute concentration of the medium is not uniform (as in the xylem, even at thermodynamic equilibrium (35, pp. 243, 244), for example), an osmotic "solute" specific free energy (within the opposite phase) related to the weighted average concentration of the solution within the phase, is employed. Localized changes of solute concentration within a phase may modify the osmotic "solute" specific free energy relations so as to alter the rate of water flow, or even its direction, through the membrane. Such thermodynamic non-equilibria would probably be rapidly adjusted, approaching those under discussion, unless the differential is continuously maintained.



energy are linear. However, single or multiple deviations from linearity occur in reality. Thus, accentuated successive changes in the thickness of the membrane (causing modifications of its modulus of elasticity), due to internal changes of volume, may lead to a "curvilinear" relation in which the second derivative of the hydrostatic specific free energy with respect to volume is negative. Contrariwise, pronounced successive changes in restriction to modification of the internal volume may lead to a "curvilinear" relation in which the second derivative of the hydrostatic specific free energy with respect to volume is positive.

In the initial state of a simplified osmotic system, as represented by the integrated endodermal system in Figure 2, in which the numbers employed in atmospheres are of magnitudes similar to those found experimentally, the fundamental equation for water movement might require, for example, the following values:

$$\text{NIF} = \text{Fs}_1 - \text{Fh}_1' \quad (11)^{11}$$

$$20.0 = 20.0 - 0 \quad (\text{see Figure 3}) \quad (11a)$$

If a difference in free energy of water molecules is partially satisfied through influx of water (endosmosis) with changes in specific free energies and internal volume of the system<sup>12</sup>, equation 11a may become, for example, after a finite interval of time,

$$13.0 = 18.3 - 5.3 \quad (11b)$$

(in Figure 3,  $\text{NIF} = 18.3 - 5.3 = 13.0$ ) and at water equilibrium

$$0 = 16.0 - 16.0 \quad (11c)$$

It is a common practice by investigators to insert manometers into the hydrostatic system of intact plants, especially species which grow to relatively great heights. It may be noted from these premises and those to follow that these instruments record the hydrostatic pressure ( $\text{Ph}_1$ ), the resultant between the intrinsic hydrostatic pressure and any possible extrinsic hydrostatic pressure or tension, existing at that time and level within the internal phase of the reference osmometer. The related hydrostatic specific free energy ( $\text{Fh}_1$ ) is thereby evaluated.

<sup>11</sup> Similar here to the fundamental equation of Meyer, where the resultant  $\text{DPD} = \text{OP} - \text{TP}$ . Also, see 6, 27, 40, 52, 57, 59, 61, 64.

<sup>12</sup> Diminution of the internal volume of the osmometer by loss of water must have the effect of increasing the internal concentration of substances to which the membrane is relatively impermeable. By mass action, reactions of which these substances are components will be accelerated, and increase in volume by influx of water will retard such reactions.



*Effect of External Solute:* If the free energy of the water molecules is lowered by the presence of solute in the external phase of the osmometer the efflux specific free energy for water is increased, and, therefore, other osmotic quantities remaining constant, the net influx specific free energy is reduced. Entry of water, to satisfy the net specific free energy tending to cause water to move inward, yields figures at equilibrium differing from those in the fundamental equation. The volume increase in the internal phase is lessened, and therefore the equilibrium value for the osmotic specific free energy related to internal solute is greater. The corresponding efflux specific free energy is higher. Here, this efflux specific free energy comprises two quantities, the osmotic "solute" specific free energy ( $F_{s_0}$ ), related to the solute concentration of the external solution (assumed to be constant), and a diminished hydrostatic specific free energy.

If the system is bathed externally by a solution with a solute concentration related to an osmotic solute specific free energy of four atmospheres, for example (in place of the initial component water), equation 11a takes the form

$$NIF = F_{s_1} - (F_{h_1}' + F_{s_0}), \quad (12)$$

where  $F_{s_0}$  is a positive  $\Delta EF$ ,

$$16.0 = 20.0 - (0 + 4.0). \quad (12a)$$

If free energy gradients are partially satisfied through influx of water, equation 12a may become

$$9.0 = 18.3 - (5.3 + 4.0) \quad (12b)$$

(in Figure 3,  $NIF = 18.3 - 9.3 = 9.0$ ) and at water equilibrium

$$0 = 16.6 - (12.6 + 4.0) \quad (12c)$$

*Effect of Internal Solute: Solute Accumulation:* Addition of solute to the internal phase of the osmometer, as represented by the fundamental equation, would increase the influx specific free energy for water by lowering the free energy of the solvent internally. At water equilibrium a greater volume is attained than would be obtained with the relatively lower internal solute concentration. The corresponding efflux or hydrostatic specific free energy is accordingly increased.

If in the system at water equilibrium, further solute is added by any means<sup>13</sup>, to the solution internally, related to an osmotic "solute"

<sup>13</sup> Influx of solute (electrolyte or non-electrolyte) through redistribution within the plant by diffusion, involving undifferentiated or differentiated cells or tissues, may ensue (52). In effect, this could occur also, for example, through hydrolysis or decondensation of solute already present in the internal phase.



specific free energy of four atmospheres, for example, equation 11c

$$\text{NIF} = F_{S_1} - F_{H_1}' \quad (11)$$

$$0 = 16.0 - 16.0 \quad (11c)$$

may become

$$\text{NIF} = (F_{S_1} + \Delta F_{S_1}) - F_{H_1}' \quad (13, \text{ see } 11)$$

$$4.0 = (16.0 + 4.0) - 16.0. \quad (13a)$$

If differences of free energy of water are satisfied, then at equilibrium

$$\text{NIF} = F_{S_1} - F_{H_1}' \quad (11)$$

$$0 = 19.4 - 19.4. \quad (13b)$$

Similarly, if to the system represented by equation 12c, in which

$$\text{NIF} = F_{S_1} - (F_{H_1}' + F_{S_0}) \quad (12)$$

$$0 = 16.6 - (12.6 + 4.0) \quad (12c)$$

further solute influx takes place, especially by metabolic accumulation from the external medium, equal to an internal increase of solute related to an osmotic "solute" specific free energy of four atmospheres, for example, the following relations arise in which

$$\text{NIF} = (F_{S_1} + \Delta F_{S_1}) - (F_{H_1}' + F_{S_0}) \quad (14, \text{ see equation } 12)$$

$$4.0 = (16.6 + 4.0) - (12.6 + 4.0) \quad (14a)$$

At water equilibrium, this becomes

$$\text{NIF} = F_{S_1} - (F_{H_1}' + F_{S_0}) \quad (12)$$

$$0 = 20.0 - (16.0 + 4.0) \quad (14b)$$

*Effect of Internal Solute Depletion:* The osmotic system at water equilibrium expressed by equation 11c (compare equation 16) may decrease with respect to its osmotic specific free energy related to the presence of internal solute, apart from a solute efflux to the external phase occasioned by possible injury, accompanied by water. Such a decrease in osmotic "solute" specific free energy might occur when either solute is translocated, without resupply from e (see Figure 2), from the inner phase of the reference cell (vacuole); or on loss of solute from the open osmometer system due to exudation; or due to a possible condensation of solute internally. The osmotic "solute" specific free energy related to the presence of internal solute would be decreased with a corresponding reduction in the net influx specific free energy, for water. If the system were at equilibrium with respect to water, a net specific free energy tending to cause water to move out of the osmotic system may become operative. In any case, as above, such a reduction in

osmotic specific free energy related to the presence of internal solute would lead to a new water equilibrium where the internal volume is decreased and the hydrostatic specific free energy lowered correspondingly. An internal solute depletion of this type equivalent to four atmospheres osmotic "solute" specific free energy, for example, would be represented by a negative change in the influx specific free energy ( $-\Delta IF$ ).

Equation 11c

$$NIF = Fs_1 - Fh_1' \quad (11)$$

$$0 = 16.0 - 16.0 \quad (11c)$$

would become

$$NIF = (Fs_1 + \Delta Fs_1) - Fh_1' \quad (15)$$

$$-4.0 = (16.0 - 4.0) - 16.0 \quad (15a)$$

tending to cause an efflux of water.

At water equilibrium, provided this condition would be realized<sup>14</sup> through an internal supply of water, the following relations would exist,

$$NIF = Fs_1 - Fh_1' \quad (15b)$$

$$0 = 12.6 - 12.6$$

*Exudation* (See paragraphs in section entitled "The Comprehensive Osmotic System of the Plant"): If the inner phase of the osmotic system is not inclosed, but rather, open, as is the xylem in the system external solution—endodermis—internal solution, a counter hydrostatic specific free energy arises resulting from the hydrostatic pressure set up due to the weight per unit cross section of the column of solution, as shown by equations 12a, 12b and 12c, whenever an inwardly decreasing specific free energy of water exists. If a net influx specific free energy is realized when the hydrostatic specific free energy, resulting from the hydrostatic pressure, is equivalent to the effective height of the inner phase (xylem)<sup>15</sup>, water will tend to exude from the upper surface as long

<sup>14</sup> Compare section entitled "Effect of External Salinity on the Open Reference System".

<sup>15</sup> Disregarding capillary rise, involving surface tension, etc. Capillarity is not specifically discussed as an influence leading to the flow of water in an osmometer system because this factor, once satisfied, remains relatively constant within limited time periods. Over longer intervals of time, it may vary in the plant, with growth; in the soil, with changes in the structure of the medium. Any effect of capillarity on the systems as analyzed will reduce the free energy of the water molecules within the osmometer phase concerned. If the inner phase is viewed as of significant capillary dimension, an influx specific free energy ( $\Delta IF$ ), resulting from a constituent internal intrinsic hydrostatic tension, will tend to cause water to move into the osmometer. Any rise of the solution within a capillary column above the hydrostatic reference level allowed by the osmotic action capacities discussed will, to this extent, increase the effective height of the inner phase.

as the required gradient of free energy of water is maintained, provided it be assumed that evaporation is negligible and the rate of water influx is greater than the water requirement of the tissues attendant on growth. Under these conditions the net influx specific free energy is termed the "specific free energy of exudation". Exudation from a decapitated plant is frequently termed "bleeding". When the flow of fluid occurs from the distal ends of xylem strands through hydathodes (tissue modifications for ease of fluid exit), the exudation is termed "guttation". For a particular system, other factors being constant, the rate of exudation is proportional to the magnitude of the net influx specific free energy (NIF). The absolute rate of water flow will equal the product of the permeability coefficient of the osmometer membrane and the net influx specific free energy gradient (Part II).

If an external solution of solute concentration equivalent to an osmotic "solute" specific free energy of 13.0 atmospheres is applied to the system expressed by equation 12a, the exudation, at an effective height equivalent to 5.3 atmospheres hydrostatic specific free energy, would be stopped and could be reversed if the osmotic specific free energy related to the presence of external solute exceeds 13.0 atmospheres.

Thus,

$$\text{NIF} = F_{s_i} - (F_{h_i'} + F_{s_o}) \quad (12)$$

$$16.0 = 20.0 - (0 + 4.0) \quad (12a)$$

tending to cause an influx of water with exudation; and

$$0 = 18.3 - (5.3 + 13.0) \quad (17)$$

water equilibrium; and

$$-1 = 18.3 - (5.3 + 14.0) \quad (18)$$

tending to cause an efflux of water.

*Effect of a Metabolic Specific Free Energy:* It has been suggested by some investigators (4, 5, 40, 42, 46, 49, 50, 66, 67; compare 13, 16, 54 and 58, 38 and 39) that under certain conditions water may move against the direction in which its concentration decreases, into the plant or plant cell. Such a flow would require direct utilization of respiratory energy<sup>16</sup>. This specific free energy, if such is normally effective in living plants, may be termed a metabolic specific

<sup>16</sup> An adequate supply of metabolite is essential to this rôle. Labile carbohydrate could serve here in two ways: directly as an osmotic solute, indirectly as a source of osmotic energy through metabolism. Obviously, its effectiveness, by these two means of water flow, is interconnected.



free energy, since the process at any time would be unidirectional and maintained through metabolism of the living cell or organism. Such a metabolic specific free energy might, for example, be related to an electrical potential difference across the membrane or between the external and internal phases, produced directly through solute migration or other means (5, 6; compare 38, 39, 49), or a temperature differential between the external and internal phases. It should be noted that such a metabolic specific free energy may also lead to unilateral movement of water with the direction in which its concentration decreases. In the latter case the metabolic specific free energy is merely serving to increase the rate of flow toward a water equilibrium demanded by an independently established net influx specific free energy. If a metabolic specific free energy is normally effective in tending to cause water to move against the direction in which its concentration decreases, it remains to be shown how the living organism governs its metabolic processes to direct the diffusion of solute and solvent through or across the cytoplasmic membrane at the same or separate intervals of time or space against the direction in which the concentrations decrease. Such a concept is theoretically possible, if water is considered (apart from its greater proportion in solution where it is regarded as solvent) like any other typical chemical species, discussed under the heading of solute accumulation.

Application of a metabolic influx specific free energy, associated with an apparent external increase of the free energy of the water molecules, would increase the net influx specific free energy and the rate of water flow. Entry of water to equilibrium would lead to an increase in the volume of the inner phase with a reduction in solute concentration of the internal solution, *i.e.*, a reduction of the osmotic specific free energy related to the presence of internal solute; and an increase of the corresponding specific free energy, resulting from an increase of internal hydrostatic pressure on the membrane. Under conditions of exudations, the specific free energy of exudation would be increased. In order to counteract the possible effects of such a metabolic specific free energy, application of an opposing specific free energy, or pressure of equivalent magnitude, would have to be applied. (Note the specific free energy required for plasmolysis and interruption of exudation.)

If the plant is able to tend to cause water to move against the

direction in which its concentration decreases, as discussed, then equations 11c and 12a, 16 and 17 would be modified quantitatively. A metabolic influx of water might lead to values represented by the following:

$$\text{NIF} = (\text{Fs}_1 + \text{Fm}) - \text{Fh}_1'$$

or  $\text{NIF} = (\text{Fs}_1 + \text{Fm}) - (\text{Fh}_1' + \text{Fs}_0)$ , in which if Fm represents a metabolic influx specific free energy of four atmospheres, for example, then

$$\text{NIF} = \text{Fs}_1 - \text{Fh}_1' \quad (11)$$

$$0 = 16.0 - 16.0 \quad (11c)$$

becomes

$$\text{NIF} = (\text{Fs}_1 + \text{Fm}) - \text{Fh}_1' \quad (19)$$

$$4.0 = (16.0 + 4.0) - 16.0 \quad (19a)$$

and at water equilibrium

$$0 = (15.4 + 4.0) - 19.4 \quad (19b)$$

Likewise, equations 12a, 16, and 17

$$\text{NIF} = \text{Fs}_1 - (\text{Fh}_1' + \text{Fs}_0) \quad (12)$$

$$16.0 = 20.0 - (0 + 4.0) \quad (12a)$$

$$9.0 = 18.3 - (5.3 + 4.0) \quad (16)$$

$$0 = 18.3 - (5.3 + 13.0) \quad (17)$$

will become respectively

$$\text{NIF} = (\text{Fs}_1 + \text{Fm}) - (\text{Fh}_1' + \text{Fs}_0) \quad (20)$$

$$20.0 = (20.0 + 4.0) - (0 + 4.0) \quad (20a)$$

$$13.0 = (18.3 + 4.0) - (5.3 + 4.0) \quad (21)$$

tending to cause an influx of water with exudation, and

$$0 = (18.3 + 4.0) - (5.3 + 17.0) \quad (22)$$

water equilibrium.

Obviously, under these conditions of a possible metabolic influx specific free energy of four atmospheres, an external solution of a concentration related to an osmotic "solute" specific free energy of 17.0 atmospheres, an increase equal to the metabolic specific free energy, would then be required to prevent exudation.

*Effect of a Non-metabolic Specific Free Energy:* If an imbibant, or action capacity similar to that associated with imbibition, is present in the external medium, the free energy of the water molecules is lowered externally. An osmotic, non-metabolic, efflux specific free energy related to an external imbibant ( $\text{Fnm}_0$ , a  $\Delta\text{EF}$ ) may arise also from this source, counteracting specific free energy tending to cause water to move into the plant (see later section en-

titled "Soil Effects"). Conversely, the presence of an imbiban in the internal phase of a plant osmometer, lowering the free energy of the water molecules therein, would act as a non-metabolic influx specific free energy ( $F_{nm1}$ , a  $\Delta IF$ ), tending to cause water to move through the membrane into the inner phase (28, 31, 58). Other non-metabolic specific free energy influences may include adsorption and capillarity. The latter, however, might be more logically conceived as a negative, constituent part of the intrinsic hydrostatic specific free energy within its phase.

*Effect of Suction, Applied Internally:* Although the effect of suction applied internally is not a natural situation, it is discussed here as an introduction to the effects of the natural internal tension caused by evaporation, as from the shoot of an intact plant. The over-all water movement may be modified by application of an extraneous internal or external pressure. Internally applied suction has a maximum pressure limit equivalent to atmospheric pressure. This suction is virtually an extraneously imposed external pressure equal to the reduction of the internal atmospheric pressure<sup>17</sup>. Application of suction internally, or an extrinsic hydrostatic pressure externally, increases the net influx specific free energy for water with its accompanying effects. Thus, if the osmometer height were unlimited, the influx of water to satisfy the increased net influx specific free energy would cause a decrease of the osmotic specific free energy related to the presence of internal solute (a decrease in solute concentration of the internal solution) and an increased internal hydrostatic pressure.

Subjecting the inner phase to such a reduced pressure of 0.8 atmosphere, for example, equation 16,

$$NIF = F_{s1} - (F_{h1}' + F_{s0}) \quad (12)$$

$$9.0 = 18.3 - (5.3 + 4.0) \quad (16)$$

tending to cause exudation, might become

$$NIF = (F_{s1} + \Delta IF) - (F_{h1}' + F_{s0}), \text{ or}$$

$$NIF = (F_{s1} + F_{h0}'') - (F_{h1}' + F_{s0}) \quad (23)$$

where  $\Delta IF$  or  $F_{h0}''$  is equal to the atmospheric pressure difference existing on the two sides of the membrane

$$9.8 = (18.3 + 0.8) - (5.3 + 4.0) \quad (23a)$$

tending to cause an extraneous additional exudation.

<sup>17</sup> See footnote \*, table I. The same results are obtained whether the specific free energy change is considered as a  $\Delta IF$  or as a  $-\Delta EF$ .

The rate of exudation is increased in proportion to the ratio NIF (equation 23a)/NIF (equation 16) = 9.8/9.0, provided other factors concerned in the rate of flow remain constant (Part II). If the osmometer height were unlimited and the water flow unrestricted, at water equilibrium equation 23a would assume the values

$$0 = (16.5 + 0.8) - (13.3 + 4.0) \quad (23b)$$

*Effect of Evaporation: Internal Tension:* Under normal conditions in the plant, evaporation of water from the inner phase<sup>17a</sup> of the simplified integrated osmometer may set up an internal partial solution tension when the rate of supply of water furnished by the net influx specific free energy, exclusive of any such tension, is less than the rate of evaporation. Here, instead of the limited increase in influx specific free energy accomplished through internally applied suction (see discussion in preceding section), the excess water loss by evaporation causes an unrestricted real tension accompanied by a diminished internal hydrostatic specific free energy resulting from a diminished internal hydrostatic pressure, and by an increase in the osmotic specific free energy related to the presence of internal solute (compare equations 16, 23a and 23b above with 16 and 24 below). Thus, after a finite interval of time, equation 16

$$\text{NIF} = \text{Fs}_1 - (\text{Fh}_1 + \text{Fs}_0) \quad (12)$$

$$9.0 = 18.3 - (5.3 + 4.0) \quad (16)$$

may become

$$16.0 = 20.0 - (0 + 4.0) \quad (24)$$

In this case the internal hydrostatic specific free energy may virtually be reduced to zero by evaporation. In the plant, however, the intrinsic hydrostatic specific free energy, or pressure, related to the weight per unit cross section of the inner phase of the osmometer, is seldom materially reduced, but, on the other hand, is often maintained at or near its maximum value. When the rate of evaporation from the inner phase of the plant osmometer plus water required by growth exceeds the rate of water supply across the semipermeable membrane, the internal hydrostatic specific free energy (an efflux specific free energy) is the net result of two

<sup>17a</sup> As evaporation occurs from a fluid column, the liquid-gas interface will recede. However, when this recession of the boundary is restricted by a perforate septum (e.g., cellulose), evaporation may give rise to a partial solution tension within the liquid phase, when the rate of supply of water furnished by a net influx specific free energy, exclusive of any such tension, is less than the rate of evaporation (28).



scalars, the intrinsic hydrostatic specific free energy and the extrinsic hydrostatic specific free energy decrement, *i.e.*, an intrinsic hydrostatic specific free energy resulting from an intrinsic hydrostatic pressure (due to the weight per unit cross section of the liquid column) diminished by an extrinsic hydrostatic specific free energy decrement, resulting from an internal partial solution tension (due to an internal negative pressure of internal extraphasic origin). In equation 24, therefore, the hydrostatic specific free energy,  $F_{h1}$ , is a net quantity equal to  $5.3 - 5.3 = 0$ , *i.e.*,  $F_{h1}' + (-F_{h1}'')$ . If the evaporation rate were to continue to increase beyond the conditions imposed by equation 24, a hydrostatic tension would arise actuating an influx specific free energy tending to cause water to move into the inner phase of the osmometer. The following relations may then be obtained:

$$NIF = (Fs_1 + (-F_{h1})) - (Fs_o) \quad (25)$$

$$18.4 = (20.8 + 1.6) - (4.0) \quad (25a)$$

Since the height of the water column has not materially changed, the intrinsic hydrostatic specific free energy remains at approximately 5.3 atmospheres. The internal hydrostatic "negative" specific free energy,  $(-F_{h1}) = 1.6$ , antiphasically effective, is then the result of the difference  $5.3 - 6.9$ , *i.e.*,  $F_{h1}' + (-F_{h1}'')$ . In Figure 3,  $(-F_{h1}) = 1.6$  is obtained from the coordinates  $(0 - (-1.6))$ , and  $Fs_o = 4.0$  from  $(2.4 - (-1.6))$ .

In equation 16 the net influx specific free energy was hypothetically moving water to an effective height commensurate with 5.3 atmospheres hydrostatic specific free energy, as an exudate. The extrinsic hydrostatic specific free energy decrement, resulting from an internal partial solution tension due to evaporation of water from the shoot, increases the net specific free energy tending to cause water to move inward through the system. However, the exudation of fluid from the surface of the inner phase apparently soon ceases when evaporation occurs. This relation will be obtained when the rate of evaporation plus water required by growth exceeds the rate of influx of water; then  $-F_{h1}'' > 0$ . The specific free energy of exudation is equal to the net influx specific free energy in the absence of an extrinsic hydrostatic specific free energy decrement, resulting from an internal partial solution tension. The action capacities causing exudation are equally operative under conditions of rapid water loss from aerial organs by evaporation. The

latter situation is merely characterized by the rate of evaporation exceeding the rate of water supply through the osmometer membrane. The movement of water, primarily through the tension, *i.e.*, the extrinsic hydrostatic specific free energy decrement, set up by evaporation (caused by reduced aerial vapor pressure), is accompanied by increased imbibitional specific free energy and increased capillarity associated with the forces of cohesion between water molecules, and are here a part of the tension expressed as an extrinsic hydrostatic specific free energy decrement,  $(-Fh_1'')$ , resulting from an internal partial solution tension.

*The Individual Cell, Plasmolysis, etc.:* Considering a reference cell system alone, many of the factors previously discussed come into action in the movement of the water from the external medium to the vacuole. The internal hydrostatic pressure is here expressed by turgor. When the osmometer is an inclosed system, the internal evaporation factor is excluded. For the isolated cell system in its initial state, the relations possibly involved in water transfer to the vacuole may be expressed as

$$NIF = Fs_1 - Fh_1' \quad (11)$$

$$20.0 = 20.0 - 0 \quad (11a)$$

or as

$$NIF = (Fs_1 + Fm) - (Fh_1' + Fs_o) \quad (20)$$

$$20.0 = (20.0 + 4.0) - (0 + 4.0) \quad (20a)$$

On entry of water to completely satisfy differences of free energy of water, these equations would assume the relations

$$0 = 16.0 - 16.0 \quad (26, \text{ see } 11c)$$

and  $0 = (16.0 + 4.0) - (16.0 + 4.0)$ , respectively. (27)

To cause plasmolysis, the water content of the internal phase would have to be reduced, through application of an efflux action capacity, to such a value that the internal hydrostatic specific free energy would be diminished to zero. As a consequence the osmotic specific free energy related to the presence of internal solute (or the solute concentration of the internal solution) would be increased. For the isolated reference cells just discussed, osmotic specific free energies, related to the presence of external solute, of 20.0 and 24.0 atmospheres, respectively, would be required to attain incipient plasmolysis.

Thus  $NIF = Fs_1 - (Fh_1' + Fs_o) \quad (28)$

$$0 = 20.0 - (0 + 20.0) \quad (28a)$$

$$\text{and} \quad \text{NIF} = (F_{s_1} + F_m) - (F_{h_1}' + F_{s_e}) \quad (20)$$

$$0 = (20.0 + 4.0) - (0 + 24.0) \quad (29)$$

*The Individual Cell: Relationships in a Tissue:* If the reference cell is surrounded by other cells, as in a tissue, intercellular pressure or tension, represented by a modified internal hydrostatic pressure, may be involved in the net influx of water. Under positive intercellular pressure conditions, osmotic turgidity of the encircled reference cell is attained by a smaller entry of water than where the reference cell is isolated<sup>18</sup>. On the other hand, when intercellular tensions are operative, a progressively increased internal hydrostatic tension obtains, for a similar reduction in volume of the inner phase of the reference cell. A positive intercellular pressure, resulting in an extrinsic internal hydrostatic specific free energy ( $F_{h_1}''$  a  $\Delta EF$ ), equal to four atmospheres, for example, might arise on influx of water into the cell aggregate or tissue. For the reference cell in its initial state, in equation 11a

$$\text{NIF} = F_{s_1} - F_{h_1}' \quad (11)$$

$$20.0 = 20.0 - 0 \quad (11a)$$

such a pressure would give the following relations at water equilibrium, namely,

$$\text{NIF} = F_{s_1} - (F_{h_1}' + F_{h_1}''), \text{ where } (F_{h_1}' + F_{h_1}'') = F_{h_1} \quad (30)$$

$$0 = 16.6 - (12.6 + 4.0) \quad (30a)$$

Under conditions leading to water depletion the intercellular pressure may exist as a negative pressure (intercellular tension) resulting in an extrinsic specific free energy decrement, tending to cause water to move into the reference cell. Likewise, the resultant internal hydrostatic specific free energy may assume "negative" values ( $-F_{h_1}$ ) leading through incipient cell plasmolysis to permanent injury, if water requirements are not satisfied. Incipient plasmolysis of the reference cell, when surrounded by other cells, would be accomplished by an external solution of solute concentration equivalent to that which plasmolysed the isolated reference cell. Here, equation 28a

$$\text{NIF} = F_{s_1} - (F_{h_1} + F_{s_e}) \quad (28)$$

$$0 = 20.0 - (0 + 20.0) \quad (28a)$$

would read

$$\text{NIF} = F_{s_1} - ((F_{h_1}' + F_{h_1}'') - F_{s_e}) \quad (31)$$

$$0 = 20.0 - ((0 + 0) - 20.0),$$

<sup>18</sup> Where the reference cell is not completely encircled, but impinged upon only in limited areas by other cells, merely minor deformations may occur. The volume relations then approach those of the isolated reference cell.

where  $Fh_1' + Fh_1'' = Fh_1$ ;

or its equivalent,

$$NIF = (Fs_1 + ((-Fh_1') + (-Fh_1'')) - Fs_0) \quad (32)$$

$$0 = (20.0 + (0 + 0)) - 20.0, \quad (32a)$$

where  $(-Fh_1') + (-Fh_1'') = (-Fh_1)$ .

Plasmolysis, beyond the state of incipience of the reference cell, to a relative volume of 0.975 would require external solutions of concentrations related to osmotic specific free energies of 22.4 and 23.4 atmospheres, respectively, for the isolated and the encircled reference cells. The equations for this would read

$$NIF = (Fs_1 + (-Fh_1')) - Fs_0, \text{ for an isolated cell,} \quad (33)$$

$$0 = (20.8 + 1.6) - 22.4;$$

and  $NIF = (Fs_1 + ((-Fh_1') + (-Fh_1'')) - Fs_0)$ , for an encircled cell, where  $(-Fh_1') + (-Fh_1'') = (-Fh_1)$ , (32)

$$0 = (20.8 + (1.6 + 1.0)) - 23.4 \quad (32b)$$

It should be noted from these considerations of the reference cell involving intercellular pressure or tension that the bounding cells impose pressures, resulting in hydrostatic specific free energies which are not linear with changes in volume. As a consequence, positive or "negative" internal hydrostatic specific free energies may be represented ideally, depending upon the circumstances, by either a linear function or a sigmoid curvilinear function. In the plant as a whole both types of functions may be operative simultaneously in the two reference osmotic systems<sup>19</sup>.

<sup>19</sup> The animal cell may be treated similarly. In this case, however, we do not deal with a reinforcing cell wall and the concomitant effects of adhesion between the wall and the cytoplasmic membrane. Therefore, similar equations and diagrams are employed for the animal cell whether isolated or within a tissue, except that the mathematical relations are restricted to the first quadrant of the graph; (the plant cell may involve relations within the first, second and third). The relative volume of one, then represents zero volume of the internal phase. The same features would apply to the plant cell if the protoplast and inclusions were completely free of interconnection with the encircling wall. The animal cell does not possess a prominent discernible central vacuole (see 68.) The true internal volume cannot be ascertained. It is necessary in computations, therefore, to employ the volume of the cell as a whole which will include a non-solvent volume (including the membrane itself) occupied by lipids, proteins and other material that set up little or no antiphase osmotic "solute" specific free energy of themselves. In this case the volume at minimal hydrostatic specific free energy (obtainable only through extrapolation) will not be zero, as required for the internal phase of the osmometer, but will be a real quantity. This value will be a measure of that part of the total space of the normal system measured, represented by the non-solvent volume.



*Effect of External Salinity on the Open Reference System:* Equations 16, 17 and 18 showed that an exudation at an effective height approximating 180 feet, equivalent to 5.3 atmospheres internal hydrostatic specific free energy or pressure, could be stopped by the presence of an external solution of solute concentration related to an osmotic "solute" specific free energy of 13 atmospheres and a tendency toward water efflux where solutions of greater solute concentration are involved. As where water evaporation occurs from the shoot of a plant (see discussion of equation 24), so also here, where a tension is applied externally, the efflux of water from the inner phase of the osmometer is not accompanied by a significant reduction in the height of the water column, but more likely by a lateral reduction in diameter.<sup>19a</sup> The net result of this outward flow of water is the maintenance of the intrinsic internal hydrostatic specific free energy at approximately 5.3 atmospheres and the establishment of an internal partial solution tension, *i.e.*, an extrinsic hydrostatic negative specific free energy component. An external solution of solute concentration related to an osmotic "solute" specific free energy of 20.0 atmospheres would modify equation 17,

$$\text{NIF} = \text{Fs}_1 - (\text{Fh}_1 + \text{Fs}_e) \quad (12)$$

$$0 = 18.3 - (5.3 + 13.0) \quad (17)$$

$$\text{to read} \quad -7 = 18.3 - (5.3 + 20.0) \quad (34a)$$

tending to cause water to move outward with consequent reduction in volume of the internal phase. At water equilibrium equation 34a would become

$$0 = 20.0 - (0 + 20.0) \quad (34b)$$

in which the internal hydrostatic specific free energy is equal to  $5.3 - 5.3 = 0$ , *i.e.*,  $(\text{Fh}_1' + (-\text{Fh}_1'')) = \text{Fh}_1$ ). For the plant these conditions of water equilibrium and of zero hydrostatic specific free energy (equation 34b) would represent, in a water saturated aerial environment, the condition of incipient permanent wilting.

*Soil Effects* (compare 17, 18, 25, 33, 41, 58, 63, 65): The discus-

<sup>19a</sup> In general, the decrease in height of fluid, from the maximum effective height of the plant or organ, in an individual more or less anastomosed hydraulic duct, will probably be limited to the initial cross wall or the vertical functional length of the first apical trachea or tracheid, but may extend under some circumstances beyond these lower transverse vascular walls. In some species of angiosperms the effective reduction in the weighted average height, of a reference unit cross section, of the hydrostatic system may be of large magnitude. The intrinsic hydrostatic specific free energy will be reduced accordingly.

sion thus far has been concerned with an external medium of water or solution of unlimited volume. Where the soil represents the external phase of the plant osmometer, specific free energies of water other than those arising from an external solute concentration of solution are generally involved. This will be particularly true at soil moisture percentages below field capacity (18). These additional non-metabolic specific free energies ( $F_{nm_s}$ ) restrain the flow of water inward, or, if of sufficient magnitude, may cause the outward passage of water through the membrane. This efflux specific free energy may include action capacities associated with reduced vapor pressure, imbibition, adsorption, capillarity and the like.

If the integrated plant osmometer be considered as exposed inwardly to a water-saturated aerial environment and separated outwardly by a soil at the permanent wilting percentage or the wilting coefficient (where the solute concentration of the soil solution is assumed, for example, to be related to an osmotic "solute" specific free energy of four atmospheres), the osmotic relations for such a system at water equilibrium would be modified from equation 34b

$$NIF = F_{s_1} - (F_{h_1} + F_{s_o}) \quad (12)$$

$$0 = 20.0 - (0 + 20.0). \quad (34b)$$

to read  $NIF = F_{s_1} - (F_{h_1} + F_{s_o} + F_{nm_o}) \quad (35)$

$$0 = 20.0 - (0 + 4.0 + 16), \quad (35a)$$

or if the plant were capable of exerting a metabolic influx specific free energy of, for example, four atmospheres, then

$$NIF = (F_{s_1} + F_m) - (F_{h_1} + F_{s_o} + F_{nm_o}) \quad (36)$$

$$0 = (20.0 + 4.0) - (0 + 4.0 + 20.0) \quad (36a)$$

The plant, in these two cases, would be at incipient permanent wilting. The total efflux specific free energy due to conditions in the external medium, the soil, would equal in equation 35a,  $4 + 16 = 20.0$  atmospheres; equation 36a,  $4.0 + 20 = 24.0$  atmospheres.

Under ordinary conditions the plant normally may be exposed at the same time to both soil osmotic factors (salinity, imbibition, water depletion, *etc.*) and aerial factors (rapid evaporation from the shoot). The plant will suffer under stressed conditions, from an unfavorable net influx specific free energy or, over an extended interval of time, from an unfavorable rate of water supply due to a reduced or inadequate membrane permeability or, directly or in-

directly due to unfavorable metabolism of the organism. Provided the permeability of the membrane remains favorable and an adequate supply of water is maintained, rapid evaporation of water from the shoot should not prove particularly harmful with regard to the plant's water relation (63, p. 614). (Note its effect on the net influx specific free energy, other conditions being favorable (29)<sup>20</sup>.) The chief causes of stress are related to water deficit or non-metabolic efflux specific free energies originating in the soil, and membrane permeability. If an additional internal hydrostatic negative specific free energy, resulting from an internal hydrostatic solution tension equal to 1.6 atmospheres, for example, is produced due to excessive water loss from the internal phase of the osmometer through aerial evaporation, equations 35a and 36a would become, respectively:

$$\text{NIF} = (Fs_1 + (-Fh_1)) - (Fs_0 + Fnm_0) \quad (37)$$

$$2.4 = (20.8 + 1.6) - (4.0 + 16) \quad (37a)$$

$$\text{and} \quad \text{NIF} = (Fs_1 + Fm + (-Fh_1)) - (Fs_0 + Fnm_0) \quad (38)$$

$$2.4 = (20.8 + 4.0 + 1.6) - (4.0 + 20.0) \quad (38a)$$

*Wilting and Death:* With respect to the water relations of the plant, the coördinated functioning of its various organs is essential to optimum growth under any set of environmental conditions. Water movements are directly or indirectly dependent on the integrated state of the meristems, sorbing and eliminating, conducting, dermal, ventilating and mechanical systems. The presence of intercellular pressures may be of advantage in the maintenance of rigidity of an organ. Under conditions of water deficit, extrinsic hydrostatic negative specific free energies, resulting from intercellular tensions, may be a detriment. The physiological anatomy and internal solution concentrations of the plant are generally adapted to accord favorably with its habitat of growth. A favorable water balance is thus provided. The solute contents of cells are graded to maintain the normal functioning of the organism as an integrated whole, even under limited stress with respect to water supply and demand. Wilting, within the limits of osmotic turgidity and zero internal hydrostatic specific free energy, during limited intervals of time, may not in itself prove lethal, directly or indirectly, to the plant.

<sup>20</sup> If the diameters of the vessels are materially reduced (likewise modifying surface tension) under the internal partial solution tensions, however, the absolute rate of water flow will be significantly reduced.

Permanent wilting of a plant may occur if, over an indefinite period of time, the supply of water is insufficient to supply the needs of the organ for growth and maintenance of a positive internal hydrostatic specific free energy. It may be considered to be in the state of incipient permanent wilting if recovery from zero internal hydrostatic specific free energy is not accomplished within a reasonable interval of time, when the plant is placed ideally into a water-saturated atmosphere, assuming approximate equilibrium within the body as a whole. If the plant in this state is in a soil approaching water equilibrium within itself, the soil is considered at the permanent wilting percentage (45, pp. 257-262). Most plant species, as a general rule, will exhibit permanent wilting<sup>21</sup> at approximately the same moisture percentage of a particular soil. This observation is probably related primarily to the rapid decrease in free energy of the water in a soil as the water content of the soil decreases on passing through the wilting percentage of the soil (17, 33, 34, 45, 65; compare 25). Any differences among separate plants or species with regard to their incipient permanent wilting under the same environmental situation will be related to differences in their inherent net influx specific free energies and their relative permeabilities. Permanent wilting and irreparable injury to cells or an organ has been related to rupture of the continuity of the water conduits. Others have related death from desiccation to destructive effects of mechanical disturbances on the structure of living protoplasm. Parallel injury effects may arise from solute toxicity (41, 65).

*Significance of Transpiration:* There is no *a priori* reason for those specific free energies, which may be applied toward producing exudation, being unable to move water to the height of the tallest plant, under appropriate conditions. However, most of the available experimental data do not favor this possibility (34). The specific free energy related to internal solute is not correlated with the height of the plant or species. The specific free energies, due to the presence of internal solute, of greater magnitudes are more frequently associated with xerophytic or halophytic species. Mano-

<sup>21</sup> In some species, evident wilting (drooping or curling of leaves or organs) does not characterize severe water deficit. Change of color of the leaves or a marked decrease in the rate of growth of the plant may indicate the exhaustion of readily available water. Desiccation below incipient permanent wilting for brief periods may not result in any apparent changes in the plant. The time factor and the rate of depletion of the available moisture are important (63).



metric pressures, equivalent to hydrostatic water columns 86 feet high, have been observed from decapitated roots of black birch (*Betula lenta*) under otherwise normal conditions. Using root tips of tomato, grown atypically by the tissue culture technique, White has reported inward movement of water against manometric pressures equivalent to a hydrostatic water column 200 feet tall. The flow was such as to indicate a dependence on respiratory processes (67). The coordinated regulation of water flow into the plant, at adequate rates, probably involves all of the possible influences previously discussed, including evaporation, *i.e.*, transpiration (8, 28, 34, 45).

*Comprehensive Osmotic System of the Plant:* For simplicity of discussion, the two reference systems have been treated using values

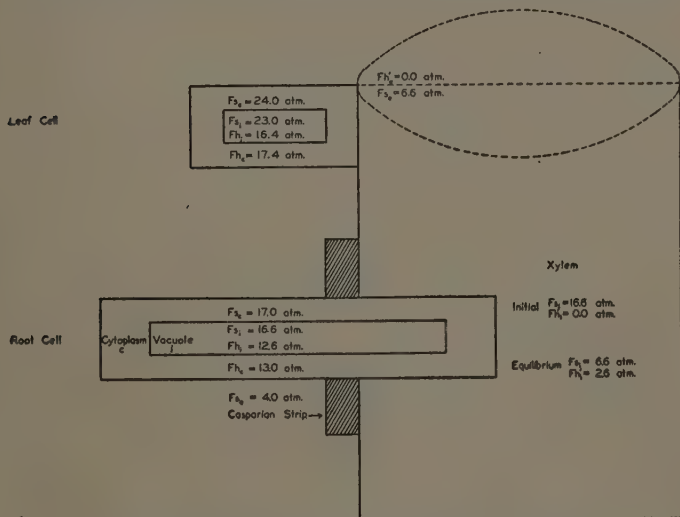


FIG. 4. The comprehensive osmotic system of the plant.

of like magnitudes for specific free energy and for volume changes. The water relations of the cytoplasm of a cell had not been analysed. The water relations of a typical leaf cell were not specifically evaluated. These concerns may be briefly discussed by values expressed in equations referring to Figure 4. The quantities related to the endodermal cell are in accord with those used earlier. The numbers recorded for the xylem, at water equilibrium, are smaller. This

assumes a larger change in volume for this internal phase, compared with that for a vacuole of a cell, on passing from minimal to maximal internal hydrostatic pressure.

*A. Water relations of the endodermal cell of the root in a water-saturated environment:*

a. At water equilibrium; cytoplasm (c); external medium (e)

$$\text{NIF} = (Fs_e) - (Fh_c + Fs_e) \quad (39)$$

$$0 = (17.0) - (13.0 + 4.0) \quad (39a)$$

b. At water equilibrium; cytoplasm (c); vacuole (i)

$$\text{NIF} = (Fs_i + Fh_c) - (Fh_i + Fs_e) \quad (40)$$

$$0 = (16.6 + 13.0) - (12.6 + 17.0) \quad (40a)$$

c. At water equilibrium; vacuole (i): external medium (e)

$$\text{NIF} = (Fs_i) - (Fh_i + Fs_e) \quad (41, \text{ see } 12)$$

$$0 = (16.6) - (12.6 + 4.0) \quad (41a, \text{ see } 12c)$$

It may be noted that this equation is equal to the sum of states represented by phases including the intermediate cytoplasm, *i.e.*, the sum of the two immediately preceding equations, as follows:

$$\text{NIF} = (Fs_e) - (Fh_c + Fs_e) \quad (39)$$

$$\text{NIF} = (Fs_i + Fh_c) - (Fh_i + Fs_e) \quad (40)$$

$$\text{NIF} = (Fs_i) - (Fh_i + Fs_e) \quad (41)$$

*B. Water relations of the integrated system of the endoderm<sup>22</sup>:*

a. Initial state; external medium (e): internal xylem medium (i).

$$\text{NIF} = (Fs_i) - (Fh_i' + Fs_e) \quad (41)$$

$$12.6 = (16.6) - (0 + 4.0) \quad (41b)$$

b. At water equilibrium (other forces excluded); external medium (e): internal xylem medium (i).

$$\text{NIF} = (Fs_i) - (Fh_i' + Fs_e) \quad (41)$$

$$0 = (6.6) - (2.6 + 4.0) \quad (41c)$$

*C. Water relations of a leaf cell in a water saturated aerial environment:*

a. At water equilibrium; cytoplasm (c): external xylem medium (e).

<sup>22</sup> The presence of an effective Casparian strip may not be considered by some to be indispensable to osmotic water movement in the integrated plant osmometer, but it certainly aids directly or indirectly in the establishment of an internal hydrostatic pressure of high order, at least under some circumstances (compare 34 and 52).

$$\text{NIF} = (\text{Fs}_e + \text{Fh}_e') - (\text{Fs}_e + \text{Fh}_e) \quad (42)$$

$$0 = (24.0 + 0) - (6.6 + 17.4) \quad (42a)$$

- b.* At water equilibrium; cytoplasm (c): internal vacuole medium (i).

$$\text{NIF} = (\text{Fs}_i + \text{Fh}_e) - (\text{Fs}_e + \text{Fh}_i) \quad (43)$$

$$0 = (23.0 + 17.4) - (24.0 + 16.4) \quad (43a)$$

- c.* At water equilibrium; vacuole: xylem. As in the preceding part A, these relations are equal to the sum of the conditions represented by phases including the intermediate protoplasm.

$$\text{NIF} = (\text{Fs}_i + \text{Fh}_e') - (\text{Fs}_e + \text{Fh}_i) \quad (44)$$

$$0 = (23.0 + 0) - (6.6 + 16.4) \quad (44a)$$

Where the aerial environment is not saturated with respect to water, a non-metabolic action capacity may arise due to evaporation, tending to cause water to move out of the leaf cell. Water relations of the xylem will be concurrently modified. Deviations from a water-saturated external medium bathing the root have been discussed hereinbefore.

In the above discussion of the comprehensive osmotic system of the plant, possible free energies related to metabolism have not been considered. The hypothetical specific free energy values used are related to the weighted average solute concentrations of the phase in question. The specific free energies related to the solute concentrations of the vacuolar solutions may not be in full accord with the viewpoints of certain investigators. Some believe that the vacuolar fluids of leaf cells may be very dilute (42, compare 16 and 68). They lay major responsibility for the influx of water on a possible metabolic specific free energy. The figures employed herein merely exemplify one opinion.

In certain commercial and experimental procedures plant fluids are obtained from wounded surfaces of aerial portions of plants as the result of scathing. The release of exudate or bleeding fluid by this means may result from an increased solute concentration at the externally effective surface. Since one or more differentially permeable membranes are probably operative between the injured surface and the internal phase of the reference osmometer, a decreasing specific free energy for water may be maintained across the intermediate surfaces by the repeated injury, through con-

tinuous disturbance of the osmotic equilibria. Generally, a concomitant hydrostatic pressure within the tracheal system has been reportedly absent. In any system water will tend to flow toward the phase or region of the lowest specific free energy. In the absence of positive hydrostatic pressures, other conditions being equal, this exudation, considered to be an efflux, would tend to occur if the lowering of the specific free energy of water due to the treatment ( $Fs_0$  or  $\Sigma EF$ ) is greater in magnitude than the concurrent total influx specific free energy ( $\Sigma IF$ ), at the same level in space.

So-called "glandular secretion" may be a natural manifestation of a similar phenomenon from uninjured tissue. In this case, a metabolic polarized movement of solute must be postulated, toward the cytoplasmic surface where the resultant exudate appears, followed or accompanied by an osmotic flow of water. A unidirectional metabolically governed flow of water has not been proven, but it is not excluded as a possibility.

The phenomenon of sap flow from red maple in early spring has been reinvestigated (55). The ultimate flow of sap is probably a mass flow of solution along lines of least resistance on removal of restraint when the hydraulic system is tapped. At times when those osmotic specific free energies which generally combine to cause exudation are ineffective (transpiration nil), temperature differences within the plant, which always exist but are considered constant elsewhere in this thesis, may play a more prominent or dominant rôle in the movement of water. Stevens and Eggert correctly suggested that as partial freezing of water occurs, water will tend to move toward this region due to a lowering of the free energy of water. This lowering is caused by two factors, lowering of the temperature *per se* and an increase of solute concentration in the remaining solution. Assuming an available source of water, *e.g.*, in the lower root regions of the soil, water will flow toward the location of partial freezing. This process will be accompanied, to some extent at least, by partial dehydration of the individual cells. The free energy difference for water is related to the degree of freezing and its accompanying effects. Ice crystallization probably proceeds in the following order: water in intercellular spaces, cell vacuoles and tracheal ducts, and finally protoplasm. In normal winter freezing this sequence is not completely accomplished, since



favorable cytoplasmic relations must be maintained for renewed growth as the season advances favorably. The release of appreciable amounts of solute (especially sugar) with the water as a flow of sap probably implies some cell destruction or at least an altered permeability and/or metabolism of cells. On thawing, an excess hydrostatic pressure will tend to cause water or solution (water and solute) to move through open channels affording a flow of sap.

*Condensation of Water at Surfaces:* The appearance of liquid at a surface may be due to two distinct phenomena which should be discriminated one from the other (compare 1). The first may be related to exudation from an organism as exudate, especially, for example, guttation fluid. This is caused by the existence of a net influx specific free energy as an over-all process by the integrated osmotic system of the plant. The second phenomenon may be related to condensation of water vapor at an exposed surface of the organism, as for example, dew. This may be caused by the lowering of the free energy of the water molecules attending energy transfer from these particles to the surface, when the surface is at or below the moisture saturation temperature. Such a condensation may be increased by the presence of solute at the interface through an enhanced lowering of the free energy of the water molecules. This amassing of fluid will occur under conditions otherwise favorable for guttation. It may also arise if the rate of amassment is greater than that of a possible rate of water flux into the plant. Continued heaping of fluid in the latter way may account, in part, for the dripping of solution from aerial organs of tree species such as *Lonchocarpus florus* and *Liveetia elegans* under conditions otherwise unfavorable for guttation. Water depositions of this kind occur in the tropical rain forest area of Latin (Hispanic) America. The overflow of collected water may or may not be assisted by capillary activity of external integuments of leaves.

*Effect of Temperature* (see 34, 44): In this treatise the temperature relations of the system have been considered to be constant. Changes in temperature under real situations will admittedly affect several of the factors involved in the flow of water. Rise in temperature will increase primarily

- (a) the free energies of the solute and solvent molecules of solutions;

- (b) the rate of evaporation (through lowered aerial vapor pressure), hence the internal partial or total solution tension, through lowering of the vapor pressure of water in the extra-phasic environment;
- (c) the rate of metabolism which may enhance, first, the rate of formation of osmotically active metabolites, and second, the release of energy for possible direct or indirect metabolic specific free energies;
- (d) the product of pressure and volume within a phase.

Temperature changes may influence the rate of water movement by modifying the viscosity of the fluid, which is a minimum at 4° C.

*Miscellaneous Effects:* Under experimental conditions still additional influences related to water flow (see also p. 27, suction effect) may be artificially applied to the osmotic system either externally or internally or both, *e.g.*, an applied electrical potential difference, or a differential mechanical or gas pressure. The constituent specific free energy ( $\Delta IF$  or  $\Delta EF$ ) would tend to move water unilaterally, similarly to that by a possible metabolic specific free energy. In the latter case, however, the osmotic influence is not artificially modified, *e.g.*, by narcosis, *etc.*

*Concluding Statements:* Heretofore in this treatise the cytoplasm of the reference cell or of the protoplasm of the integrated system has been assumed to be homogeneous in its properties and of uniform metabolism. As such, it was viewed, *in toto*, as a differentially permeable osmometer membrane<sup>23</sup>.

These relationships no doubt differ in space within the ectoplasm, mesoplasm and endoplasm, and with time. Differences in the specific free energy of solute and solvent particles within these regions would be expected to exist. If a decreasing solute concentration is maintained across the protoplasm from the ectoplasm to the endoplasm of either system, water equilibrium could be readily attained through a lowered hydrostatic specific free energy within the system as a whole. Since, in any case, the difference of osmotic

<sup>23</sup> Especially in cells in which a central vacuole is not apparent, as for example in meristems, it is possible that a dispersed system of minute osmometers may exist within the cytoplasm. The theories of Duclaux; Proctor and Wilson, and Loeb (30, p. 122; 9, p. 333) may have practical application here. It is suggested that gel walls act as membranes which surround dissolved molecules or extremely small colloidal particles. It is assumed that limited swelling occurs accompanied by elastic stretching of the gel walls in opposition to an internal hydrostatic pressure, in response to an influx of fluid caused by an established net influx specific free energy.

"solute" specific free energy through the membrane itself would be small, the net specific free energy tending to cause water to move into the integrated system would be very closely expressed by the relative osmotic "solute" specific free energies and hydrostatic specific free energies related to the external and internal phases, with the protoplasm considered as a simplified homogeneous membrane.

Operation of the integrated endodermal system as discussed herein assumed prior equilibrium within the endodermal cell. Actually, satisfaction of water deficits in the two reference systems under discussion and within a cell series may proceed simultaneously (compare 34 with 6 and 59).

In the plant as a whole, water will tend to move with the direction in which its specific free energy decreases. In any case, the tendency for water movement at any instant of time will be accurately expressed in terms of the net energy tending to cause water to move through the membrane of the osmometer, namely, the net influx specific free energy. The over-all rate of water movement (Part II) will depend on the product of the permeability of the membrane, and the net influx specific free energy gradient, in accordance with Poiseuille's or Torricelli's theorem (included in the permeability coefficient), as applied to the flow of liquids through tubes; the latter, depending upon which formula, if either, is more or less applicable (compare data of Lundegårdh, 39, and 7, p. 142).

#### EXPERIMENTAL EVALUATION OF OSMOTIC QUANTITIES

*Summary of Osmotic Relations:* A brief recapitulation of equations and reference states may be made as an introduction to an analysis of experimental methods for the estimation of osmotic values in certain type cases.

The osmotic specific free energy balance, tending to cause water to move into plants is expressed by the equation,

Net influx specific free energy =  $\sum$ Influx specific free energies—  
 $\sum$ Efflux specific free energies.

$$NIF = \sum IF - \sum EF$$

$$NIF = (IF + \Delta IF) - (EF + \Delta EF), \text{ or, for example,}$$

$$NIF = (Fs_1 + Fm) - (Fh_1 + Fs_0), \quad (20)$$

where  $Fh_1 = Fh_1' + Fh_1''$ ,  $Fm$  and  $Fs_0$  are used as possible examples of additional influx and efflux specific free energies.

NIF is the net influx specific free energy tending to cause unbound (or solvent) water to move into the internal phase of the plant osmometer.

$\Sigma IF$  is the algebraic sum of those osmotic specific free energies tending to cause unbound (or solvent) water to move across the semipermeable membrane into the internal phase of the plant osmometer; opposed to  $\Sigma EF$ .

$\Sigma EF$  is the algebraic sum of those osmotic specific free energies tending to cause unbound (or solvent) water to move across the semipermeable membrane into the external phase of the osmometer.

$F_{s_i}$  is the antiphase osmotic specific free energy related to the solute concentration internally; see  $C_i$ .

$F_{s_e}$  is the antiphase osmotic specific free energy related to the solute concentration, externally; see  $C_e$ .

$C_i$  is the internal solute concentration related to  $F_{s_i}$ .

$C_e$  is the external solute concentration related to  $F_{s_e}$ .

$F_m$  is a possible metabolic specific free energy, here inwardly directed.

$F_{h_i}$  is the internal hydrostatic specific free energy equal to the algebraic result between the intrinsic and extrinsic hydrostatic specific free energies.

Since in any biological osmotic system  $F_{s_i}$  and  $F_{h_i}$  are fundamentally involved, they have been designated as IF and EF, respectively, in the general equation.  $F_m$  and  $F_{s_e}$  may or may not apply in any specific case, and are therefore symbolized in a general way by  $\Delta IF$  and  $\Delta EF$ .

$V$  is the volume of the internal phase of the osmometer in arbitrary units (the vacuolar, or xylem and possibly intercellular space within the endodermis, is essentially implied for the two systems discussed).

$e$  and  $i$  are subscripts referring to the external and internal phases, respectively, of the reference osmometer; the regions within which the constituent influence, modifying the free energy of the component water molecules, may be imposed.

$o$ ,  $n$  and  $m$  are subscripts referring to the conditions of the osmometer at zero, intermediate normal or control, and maximal internal hydrostatic specific free energy (*i.e.*, that attainable when the external medium is water).

$\Delta$  is a symbol for a finite change in any osmotic value.

At zero internal hydrostatic specific free energy (*e.g.*, a cell at incipient plasmolysis) :

$$NIF_o = (Fs_{io} + Fm_o) - (Fs_{eo}) \quad (45)$$

since  $Fh_i$  is zero, here. At water equilibrium  $NIF_o = 0$ , therefore

$$Fs_{eo} = (Fs_{io} + Fm_o) \quad (46)$$

At normal, or control internal hydrostatic specific free energies:

$$NIF_n = (Fs_{in} + Fm_n) - (Fh_{in} + Fs_{en}) \quad (47)$$

At water equilibrium  $NIF_n = 0$ , therefore

$$Fs_{en} = (Fs_{in} + Fm_n) - Fh_{in} \quad (48)$$

or

$$Fh_{in} = (Fs_{in} + Fm_n) - Fs_{en} \quad (49)$$

At maximal internal hydrostatic specific free energy (external phase pure liquid, water) :

$$NIF_m = (Fs_{im} + Fm_m) - (Fh_{im}) \quad (50)$$

since  $Fs_{em}$  is zero, here. At water equilibrium  $NIF_m = 0$ , therefore

$$Fh_{im} = (Fs_{im} + Fm_m) \quad (51)$$

*Procedures for the Estimation of Osmotic Quantities (see 57):*

There are two general means of approach to these values; the first, dealing with water equilibria, involves the following techniques:

1. Measurement of the internal volume of the osmometer at zero, normal and maximal internal hydrostatic specific free energy (resulting from the corresponding pressure) of the system.
2. Determination of the solute concentration of the external medium of the osmometer at water equilibrium, for the system at zero and normal internal hydrostatic specific free energies; and/or the related (antiphasically effective) osmotic specific free energy, by calculation (see 10), or by cryoscopic (14, 22, 24) or vapor pressure (21) methods.
3. Determination of the osmotic "solute" specific free energy related to the presence of solute in the internal medium<sup>24</sup> of the osmometer, by cryoscopic, or vapor pressure, or hydrostatic pressure (22, 48) methods.

<sup>24</sup> By determining the specific conductance (volume conductivity, in reciprocal Ohm—centimeters, *i.e.*, mhos per centimeter,  $L$ ) of the fluid, the ratio of the specific conductance to the comparable freezing point depression (in degrees absolute lowering,  $\Delta T_f$ ), can be obtained. This ratio ( $L/\Delta T_f$ ) is an expression for the relative abundance of electrolytes as compared with the total solutes (electrolytes and non-electrolytes) present in the medium (23).



4. Estimations of certain other osmotic quantities by a special technique (2); see case B, following.

By these means, values may be obtained for most of the quantities desirable for an inspection of the system as a whole, whether a reference cell or the integrated (open inner phase) plant osmometer.

The second approach is of value only for the integrated plant osmometer system. Here, non-equilibria for water are primarily concerned. The following techniques are involved:

1. Measurement of the relative rates of flow of liquid across the semipermeable membrane, generally made at or approaching zero internal hydrostatic specific free energy as modified by various solute concentrations in solutions applied to the external phase.
2. Determination of the solute concentrations of the external medium, and/or the related osmotic specific free energies (by methods as in item 2, above), at various rates of liquid flow as measured in item 1, immediately preceding.
3. Determination of the osmotic "solute" specific free energy related to the presence of solute in the internal medium of the osmometer as in item 3, above.

Any series of operations involves assumptions of various validity. The correctness of any assumption will depend upon a number of factors, especially on the system involved and the experimental approach. Some of the more important assumptions may be listed for the two major systems. The validity of each assumption may be reasoned from a detailed examination of the particular experimental situations involved in any set of data. Following are assumptions associated with the reference cell as the osmotic system:

1. Time is infinitesimal.
2. Temperature and metabolism (compare 5) are constants.
3. There is no movement of solute across the semipermeable membrane. The permeability of the membrane is constant to either solute or solvent with time.
4. The elastic modulus of the membrane is constant in space and time (compare 1).
5. The measured volumes of the internal phase of the osmometer are that of the reference cell vacuole at water equilibria. Where tissues are employed the cells are uniform, acting as individual entities, *i.e.*, intercellular pressures or tensions are absent. (Note,

measurements of tissue lengths involve errors embodied in assumptions 4 and 5.)

6. Incipient plasmolysis is accurately estimated. Adhesion between cytoplasm and wall is nil.

7. The determined solute concentration of the external medium is its weighted average concentration, bathing the membrane externally, at equilibrium (see footnote 10). The osmotic specific free energy related to the presence of external solute is accurately determinable<sup>25</sup>. The concentration of the external medium is constant with time.

8. The osmotic specific free energy related to the presence of solute in the internal medium is accurately determined through the analytical technique and mathematical procedure employed (see footnote 25). (Note, composite expressed sap from whole cells involves obvious aberration, involving vacuolar, cytoplasmic and extra-cellular fluid associated with non-solvent (bound) water and modified solute effects.) Modification of the solute concentration with proportional change in volume is calculated from the ratios of the volumes to that of concentrations, not to that of osmotic "solute" specific free energies.

9. The osmotic specific free energies related to the presence of external and internal solute, are equal at osmotic water equilibrium, but only when the internal hydrostatic specific free energy or pressure is zero and if metabolic specific free energy is nil.

10. Hydrostatic specific free energy changes, internally, are proportional to the corresponding changes in volume of the internal phase of the osmometer. The hydrostatic specific free energy internally is zero at incipient plasmolysis.

11. The hydrostatic specific free energy, externally, is nil.

Following are assumptions associated with the integrated reference osmotic system:

1. Time is infinitesimal.

2. Temperature and metabolism are constants.

3. The permeability of the osmotic membrane is constant to either solute and solvent with time.

<sup>25</sup> This implies that the anti-phasically effective osmotic "solute" specific free energy mathematically obtainable from the related solute concentration in solution is accurately expressed by means of van't Hoff's equation (see equations 3, 3a and 3b), and that any essential, specific free energy, correction coefficient (see footnote 6) is accurately known and applied for the conditions experimentally imposed.

4. The rates of fluid flow through the semipermeable membrane into the inner phase of the osmometer, as modified by various solute concentrations applied to the external phase, are constant with time for each set of conditions.

5. The concentration of each of the solutions applied externally is constant with time. The determined solute concentration of the external medium is its weighted average concentration bathing the membrane externally (see footnote 10). The osmotic specific free energy related to the presence of external solute is accurately determinable (see footnote 25).

6. The osmotic specific free energy related to the presence of solute in the internal medium is accurately determined through the analytical technique and mathematical procedure employed (see footnote 25). This quantity is the weighted average value for the solution bathing the membrane internally (see footnote 10), corresponding to the determined rate of flow of fluid.

7. The hydrostatic pressures, internally and externally, are nil.

Obviously biological systems differ from these assumptions in varying degree. An observed or calculated osmotic quantity is the algebraic result of the possible deviations from the true value. The importance of these possible deviations can be realized, for example, from the controversy over the reality or non-existence of a metabolic specific free energy (active, vital, non-osmotic pressure) as a possible action capacity normally tending to cause water to move into plants.

*Osmotic Quantities of a Cell: Two Type Cases: Case A:* For those unfamiliar with the procedure followed in arriving at the values recorded in the literature for osmotic phenomena, a type case may be presented based on a recent study of Lyon (40), following essentially the plan of Ursprung (61). (Compare 4, 13, 42, 56, 66.)

1. The tissue volumes are obtained, from optical measurements, at zero (incipient or limiting plasmolysis of 50% of the cells), normal and maximal internal hydrostatic specific free energies (or corresponding pressures) of the cell. Thus

$$\begin{array}{llllll} V_o = 2106 \text{ in arbitrary units, or } V_o = 1.000 \text{ in relative units} \\ V_n = 2380 \text{ " " " " } V_n = 1.130 \text{ " " " " } \\ V_m = 2860 \text{ " " " " } V_m = 1.356 \text{ " " " " } \end{array}$$

2. The sucrose solution (plasmolyte) concentration necessary to just plasmolyse 50% of the cells in the tissue is observed. Thus  $C_{eo} = 0.70$  Molar (mols per liter of solution).

3. The antiphasic osmotic "solute" specific free energy related to the plasmolysing solution is estimated from item 2 by calculation (see 10; and item 7, page 48) or by means of conversion data (see 47, 48, 62). Thus  $C_{eo} = 0.70$  M sucrose, is related to  $F_{seo} = 21.5$  atmospheres. (Here, as in the calculations of Lyon (40), the conversion data of Molz (47) were used.) Where cryoscopic analyses are performed, the osmotic "solute" specific free energies are determined directly (24).

4. By observing the sucrose solution concentration as a bathing medium necessary to maintain the normal volume, or weight (see 46, 57), relationships within the tissue,  $C_{en}$  and  $F_{sen}$ , can be similarly estimated (see items 2 and 3). Lyon estimated  $C_{en} = 0.32$  M and the related  $F_{sen} = 8.7$  atmospheres.

5. At equilibrium between the vacuoles of the cell tissue and the plasmolysing solution the tissue could be treated by freezing and sap expression and the osmotic "solute" specific free energy, related to the solute concentration of the composite sap, determined by cryoscopic, vapor pressure or *in vitro* osmotic technique, as a measure of the antiphasic osmotic "solute" specific free energy related to the presence of internal solute. This estimate may be open to serious error due to admixture of fluids and modified by bound water effects (13). Thus  $C_{io}$  and  $F_{sio}$  could be found. Using tissue at normal internal hydrostatic specific free energy and at maximal internal hydrostatic specific free energy,  $C_{in}$  and  $C_{im}$ , and  $F_{sin}$  and  $F_{sim}$  can be similarly obtained.

On the *a priori* assumption that an inwardly directed metabolic specific free energy does not exist (which is not justified, but may yield an approximate measure of such a pressure from subsequent calculations) Lyon set  $F_{seo} = F_{sio}$  and  $F_{sen} = F_{sin}$  (see items 3, 4 and 5). Assuming that the internal solute concentration is inversely proportional to the change in volume of the inner phase,  $C_{in}$  and  $C_{im}$  can be calculated. Thus

$$\frac{C_{in}}{C_{io}} = \frac{V_o}{V_n} \text{ or } C_{in} = C_{io} \times \frac{V_o}{V_n} = 0.70 \times \frac{2106}{2380} = 0.620 \text{ Molar,} \quad (52)$$

and

$$C_{im} = C_{io} \times \frac{V_o}{V_m} = 0.70 \times \frac{2106}{2860} = 0.515 \text{ Molar.} \quad (52a)$$

By conversion,  $F_{sin} = 18.5$  atmospheres, and  $F_{sim} = 14.8$  atmospheres. The value  $F_{sio}$  can not be calculated directly from  $F_{sio}$

by using the quotient of the volumes as a factor (compare 40 and 61), because the related osmotic "solute" specific free energy changes at a greater rate, represented by a hyperbolic function, than does the concentration (61; 57, pp. 51-56).

6. The internal hydrostatic specific free energy at maximal turgor is computed from the equal and opposing action capacity, the osmotic specific free energy related to the presence of internal solute, at maximal turgor. Thus,  $Fh_{im} = Fs_{im} = 14.8$  atmospheres. The internal hydrostatic specific free energy at minimal turgor or turgidity is zero by definition. Thus,  $Fh_{io} = 0$  atmospheres. In the absence of intercellular (tissue) pressure or tension, the internal hydrostatic specific free energies at normal turgor can be computed from the proportional change of internal hydrostatic specific free energy with internal volume. Thus

$$\frac{Fh_{in} - Fh_{io}}{Fh_{im} - Fh_{io}} = \frac{V_n - V_o}{V_m - V_o}, \text{ but since } Fh_{io} \text{ is zero, then}$$

$$Fh_{in} = Fh_{im} \times \frac{V_n - V_o}{V_m - V_o} = 14.8 \times \frac{2380 - 2106}{2860 - 2106} = 5.4 \text{ atmospheres. (53)}$$

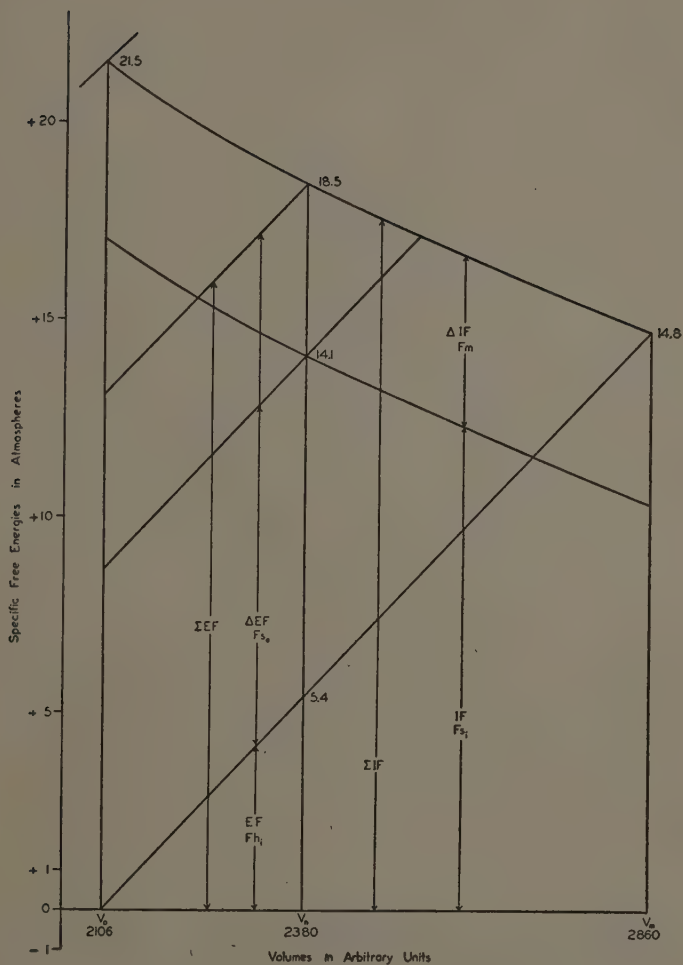
7. It was assumed (under item 5, which see) that a possible inwardly directed metabolic specific free energy was non-existent.  $Fs_{en}$ , under item 4, was estimated to be 8.7 atmospheres. (In Figure 4,  $Fh_{in} + Fs_{en} = \Sigma EF$  or  $5.4 + 8.7 = 14.1$ ). However, from the following calculation (data from items 5 and 6)

$$Fs_{en} = Fs_{in} - Fh_{in} = 18.5 - 5.4 = 13.1 \text{ atmospheres.}$$

The difference of 4.4 atmospheres between these values (8.7 atm. and 13.1 atm.) leads to the conclusion that an additional osmotic action capacity is operative in the system. This action capacity may obviously be realized as the metabolic specific free energy assumed to be non-existent in the earlier analysis of these data (see under item 5). The magnitude is an approximation from the mathematical process involved. These data, on the basis of the present scheme, are shown graphically in Figure 5. A more direct approach to this quantity would have been from determined values of the osmotic "solute" specific free energies related to the solute concentrations in the fluids from the external and internal phases of the osmometer system at minimal turgor and water equilibrium.

Then  $NIF_o = (Fs_{io} + Fm_o) - (Fh_{io} + Fs_{eo})$  where  
 $NIF_o$  and  $Fh_{io}$  are zero,  $Fs_{eo} = (Fs_{io} + Fm_o)$  or





## OSMOTIC RELATIONS OF A POTATO TUBER CELL

$$[NIF = \Sigma IF - \Sigma EF = (F_{s_2} + F_m) - (F_{h_1} + F_{s_2})]$$

FIG. 5. Water relations of a plant cell. The fundamental data were obtained, with slight modification (see text), from Lyon (40). The hyperbolic curves representing  $IF$  (*i.e.*,  $F_{s_1}$ ) and  $\Delta IF$  (*i.e.*,  $F_m$ ) are drawn parallel. This assumes that the metabolic specific free energy is constant under the experimental conditions.

$Fm_o = Fs_{eo} - Fs_{io}$ ; in other words, a positive difference between the determined values of the osmotic "solute" specific free energies, as written, would suggest in reality a possible inwardly directed metabolic specific free energy tending to cause water to move into the biological osmotic system.

*Case B:* The ingenious apparatus designed by Arens (2) would appear to afford exceptional opportunities for the measurement of certain osmotic quantities. By this technique a band of a single *Nitella* cell can be fixed in an impermeable metal collar. The arrangement may be likened to an individual endodermal cell encircled by its Casparian strip. The tractable terminal sections are enclosed by spaces which may be occupied by gases or liquids. In the latter situations this osmometer may be experimentally observed under such differential osmotic relations as may be applied to the opposite poles of the cell. The cell as a whole thus acts like a living semipermeable membrane, similar to the integrated osmotic system discussed for the entire plant. The system may also be viewed as a single cell; therefore, the arrangement affords systems of an isolated cell and an integrated osmometer at one and the same time.

The designer has subjected his experimental cell to an artificial situation where at least one of the spaces surrounding the free ends of the organ is filled with gas under pressure. The turgidity of the cell is observed by the angle of declination from the horizontal presented by the unrestricted portions of the filament. The flow analysis now proposed allows the experimental procedures of this case to be critically evaluated. At water equilibrium, the cell may, for example, assume the values (see Figure 3)

$$\begin{aligned} NIF &= \sum IF - \sum EF \\ NIF &= (Fs_i) - (Fh_i) \\ 0 &= (16.0) - (16.0). \end{aligned} \quad (54, \text{ see } 11c)$$

On evaporation of water from this cell, when subjected to a gaseous current of low moisture content in the external environment, the relative vacuolar volume might decrease from 1.5 to 1.085. This loss of turgor may then be represented by changes in the osmotic quantities to the values

$$13.0 = (18.3) - (5.3) \quad (55)$$

A differential of gas pressure is then rapidly applied (compare section entitled "Miscellaneous Effects") to the free portions of the

cell to restore the angle of declination, due to wilting, to zero. Accompanied by no increase in the volume of the cell vacuole, the net influx specific free energy would then approach zero, *i.e.*,

$$\begin{aligned} \text{NIF} &= (F_{s1}) - (F_{h1}) \\ 0 &= (18.3) - (18.3) \end{aligned} \quad (56)$$

$F_{h1}$  is equal to the sum of two specific free energies, an intrinsic and an extrinsic internal hydrostatic specific free energy, where

$$F_{h1} = F_{h1}' + F_{h1}'' = 5.3 + 13.0 = 18.3 \text{ atmospheres.}$$

The extrinsic internal hydrostatic specific free energy ( $F_{h1}''$  or  $\Delta EF$ ) arises in opposition to the externally applied pressure. In other words, the extrinsic internal hydrostatic specific free energy, here equal to 13.0 atmospheres, is the result of the applied gas pressure measured by the manometer or gas gauge.

Experimentally observed manometric pressures of 3–10 atmospheres have been reported (2). In the absence of a possible metabolic specific free energy, this osmotic quantity is also a measure of the net influx specific free energy for water when the cell was wilted. Contrary to report (2), this pressure is not a measure of the initial internal hydrostatic specific free energy of the cell at maximal turgor. This was actually 16.0 atmospheres (see equation 54).

*Osmotic Quantities of an Integrated System: Two Type Cases:*  
*Case A:* The following data were selected from van Overbeek (40) in which the osmotic specific free energy balance was observed for decapitated plants at water equilibrium (compare 16 and 39).

1. The osmotic specific free energy related to external solute was calculated from cryoscopic measurements of the external medium which just prevented water movement through the membrane of the plant. The  $F_{s_{eo}}$  thus found was 1.40 atmospheres.
2. The osmotic specific free energy related to the presence of internal solute was calculated from cryoscopic measurements of the exudate when the roots were bathed, over short intervals of time, with water. The  $F_{s_{1o}}$  thus found was 0.40 atmosphere.
3. Since the system was observed essentially at zero internal hydrostatic specific free energy, and at water equilibrium, a difference between these two quantities,  $F_{s_{eo}}$  and  $F_{s_{1o}}$ , may be

ascribed to an inwardly directed metabolic specific free energy. By difference this metabolic specific free energy is found to equal 1.00 atmosphere. (*i.e.*,  $F_{m_0} = F_{s_{e0}} - F_{s_{i0}} = 1.4 - 0.4 = 1.0$  atm.) In a similar type of experiment by Eaton (16) a metabolic specific free energy could not be realized, *i.e.*, the difference between  $F_{s_{e0}}$  and  $F_{s_{i0}}$  was not significant. The osmotic analysis at water equilibrium was preferred over methods involving the rate of bleeding, in that changes in the water permeability of the root cells are not considered to affect these specific free energy determinations (compare case B, below).

*Case B:* The following data were selected from Sabinin (54) and Litvinov (36) in which the osmotic quantities were derived from the relative rates of bleeding from decapitated plants subjected to external media of known solute concentration (compare 39).

In the following equations similar symbols are employed as before. In addition, initial velocities of exudation are expressed by  $v_m$ ,  $v_n$  and  $v_o$  representing, respectively, the velocities when the system is exposed to external phases of water, hypoosmotic and isoosmotic media. The character  $k$  is a permeability factor. This coefficient of proportionality ( $k$ ) is a constant if the rate of exudation is not changed by a subsequent transfer of the root system into water following an experimental transfer from water to a solution. During brief intervals of time (less than 30 minutes), exudation velocities did not vary significantly (54).

Litvinov (36) made use of the following types of equations, assuming constancy of  $k$ , and  $F_{s_1}$  and  $F_m$ , and obtained the corresponding figures, in one experiment:

$$v_m = (k_m) \cdot (NIF) \quad (57)$$

$$v_{n1} = (k_{n1}) \cdot (NIF) \quad (57a)$$

$$\text{and then} \quad v_{n2} = (k_{n2}) \cdot (NIF) \quad (57b)$$

$$NIF = \frac{v_m(F_{s_{en1}})}{v_m - v_{n1}} = \frac{(14) \times (6.4)}{(14) - (3.5)} = 7.8 \text{ atmospheres} \quad (58)$$

$$\text{or} \quad NIF = \frac{v_m(F_{s_{en2}})}{v_m - v_{n2}} = \frac{(14) \times (7.6)}{(14) - (0.5)} = 7.9 \text{ atmospheres.} \quad (58a)$$

When the velocities are compared, where the root system is alternately bathed by two solutions of dissimilar concentrations (rather

than in the one case by water), use is made of the following relation, where

$$\text{NIF} = \frac{(v_{n1}) \times (F_{S_{en2}}) - (v_{n2}) \times (F_{S_{en1}})}{v_{n1} - v_{n2}} \quad (59)$$

$$\text{NIF} = \frac{(3.5) \times (7.6) - (0.5) \times (6.4)}{(3.5) - (0.5)} = 7.8 \text{ atmospheres.} \quad (59a)$$

The osmotic "solute" specific free energy, related to the solute concentration of the exudate when the roots were bathed in water, was obtained cryoscopically. This was compared with the net influx specific free energy determined independently. Placing figures expressed in atmospheres, cited from Table 3, p. 51 (36), into the appropriate equation:

$$\text{NIF} = (F_{S_1} + F_m) - (F_{h_1} + F_{s_0}) \quad (60)$$

$$\text{Where } F_{h_1} \text{ and } F_{s_0} \text{ are zero, then } F_m = \text{NIF} - F_{S_1}. \quad (61)$$

The following relations are obtained:

$$-0.25 = 0.43 - 0.67$$

$$-0.01 = 0.35 - 0.36$$

$$-0.04 = 0.38 - 0.42$$

$$+0.07 = 1.53 - 1.46$$

The results would suggest the absence of any significant metabolic specific free energy in the movement of water in these plants. It should be noted that negative evidence of this sort does not constitute proof of the non-existence of metabolic specific free energies in biological systems as a whole. Small positive figures, although suggestive of the presence of such action capacities, may be related to errors in technique.

#### SUMMARY

The fundamental principles of osmosis are reviewed. A scheme is presented for mathematically dealing with the osmotic quantities, expressed as osmotic specific free energies, and graphically representing the same in a diagram with appropriate coordinates. Theoretical cases are introduced, utilizing various osmotic action capacities which possibly control water movement between the plant and its environment. Four typical sets of data are analysed by the present scheme and limitations discussed.

A survey is made of methods currently employed in obtaining various osmotic quantities in plants.

The controversy as to the reality or non-existence of metabolic



specific free energies (variously termed active, vital or non-osmotic pressures) must await more critical experimentation.

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